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1958 Contributions

44. Hiatt, Robert W. 1958. Factors influencing the distribution of corals on the reefs of Arno Atoll, Marshall Islands. 8th Pacific Sci. Congress.

49. Gosline, William A. 1958. The nature and evolution of the Hawaiian inshore fish fauna. Proc. 8th Pacific Sci. Congress.

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FACTORS INFLUENCING THE DISTRIBUTION OF CORALS ON THE REEFS OF ARNO ATOLL, MARSHALL ISLANDS[1]

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Introduction

This paper presents a small but significant facet of a broad investigation into the marine ecology of Arno Atoll, Marshall Islands, which, in turn, is a portion of a comprehensive study of the atoll by a team of scientists representing several disciplines including geology, geography, hydrology, climatology, marine ecology, botany, entomology, agriculture, vertebrate zoology, and anthropology. The objective of the integrated investigation was to provide a broad basis for learning the nature of life processes in these self-contained microcosms so that a coherent and understandable picture of a limited tropical total environment would emerge.

Although the writer was engaged in studying many ecological aspects of the reef environment about the atoll, one compelling feature was the recognition that, regardless of the geographical location about the atoll, the reef tracts were roughly zoned parallel to the reef margin. Oftentimes the zones were characterized by certain organisms which dominated the area; elsewhere the parallel bands were depositional flats, or frequently were solid and pavement-like. In any case it was apparent that the distribution and abundance of virtually the entire assemblage of organisms which dwell upon these coral reefs were intimately associated with and influenced by the various reef facies present. Because of these relationships a systematic collection of data along transects across both the sea and lagoon reefs was undertaken to interpret more precisely the underlying causes of zonation. During this study very wide fluctuations in salinity, far greater than any reported previously in the literature, were discovered. Wide differences in salinity were noted between high and low tides and between the sea and lagoon reefs. Since these variable salinity gradients appeared to have no direct relation to rainfall, it was obvious that the only source of fresh water could be the ground-water held within the islands and islets.

[1] Contribution No. 44, Hawaii Marine Laboratory, in cooperation with the Department of Zoology and Entomology, University of Hawaii.

By working closely with the hydrologist of the party an explanation of this phenomenon became apparent, and it is this environmental factor which is analyzed in greatest detail in this report. However, because zonation on coral reefs doubtless results from the interaction of many environmental factors, several of these which are considered important on Arno reefs are stressed.

Only corals are used as key animals in defining various aspects of zonation on these reefs because they are fixed in position and each species possesses a relatively narrow ecological valence. Thus the distribution of certain types of corals may be used as an indication, of limiting or inhibiting factors present across the reef tract. Important too is the fact that they may be considered quantitatively, both as regards species and population densities.

Location and General Description of Arno Atoll

Arno Atoll, lying in the southern Marshall Islands at approximately 172°E. and 7°N., is in the northeast trade wind belt and for most of the year in the path of the North Equatorial Current. During the summer months the Equatorial Counter Current swings northward, deflecting the North Equatorial Current, and probably influences the atoll with its eastward flow. Ocean water temperatures do not fluctuate more than 1 degree above or below 28°C. The temperature, clarity and agitation of the surface waters are optimum for coral growth, but the planktonic food supply, despite the apparent favorable position at the convergence of major ocean currents, is less than in areas further to the southwest, so coral growth is somewhat below the potential maximum, yet must be classified as flourishing.

Arno is rectangular, rather than a circular, atoll with two extensions or "horns," one extending to the north about 5 miles from the north center of the rectangle, and the other extending about 7 miles northeastward from the east corner (Fig. 1). The total area enclosed by the seaward reefs and lagoons is about 147 square miles. The surface reefs cover about 16 square miles and on them 133 islands and islets form about 5 square miles of dry land. Two deep passes and 3 shallow ones interrupt the continuous seaward reefs around the atoll. The total circumference of the seaward reefs is about 65 miles.

The islands and islets have been built by the forces of winds and waves from materials derived from the living reefs. They are accumulations of loose material partly held together by a framework of beach conglomerate to seaward, beach sandstone lagoonward and interior island sandstone. Surface processes and changes are everywhere in

evidence about the atoll, and frequently vast changes are observed, primarily on the lee side of the atoll, which have resulted from occasional great typhoons.

Reef types at Arno Atoll have been briefly described by Wells (1951) and classified by him following the scheme for designating seaward reef types set forth by Tracey, Ladd and Hoffmeister (1948). These are shown in Figure 1.

The lagoon reefs are less continuously developed than the seaward reefs. The best development on the lagoon side occurs along the west, south and north sides of the atoll. The only sizeable development of leeward lagoon reefs on the north or windward side of the atoll is in the vicinity of the two deep passes, Tutu and Tak-lib. Coral knolls or patch reefs are numerous in the lagoon and Wells surmises that their distribution is less than fortuitous, inasmuch as the clusters appear to bear some relation to the passes through the windward rim of the atoll.

The dominant type of seaward reef (Type IA), characterized by strong grooves on the seaward slope which do not cut the low algal ridge at the margin, extends almost uninterruptedly from the west side of the North Horn southward around the lee side of the atoll to the eastern edge of the East Horn. The inner half or more of the reef platform is flat, almost barren, and supports coral growth only in occasional depressions. Blanketing this reef flat is an extensive growth of a brown alga (*Microdictyon okamurai*) on the fronds of which live vast quantities of the foraminiferan *Calcarina*. Here and there are many large, securely attached rock oysters (*Chama*). Wells has termed this zone *Calcarina-Chama*. On the gradually sloping outer third of the reef flat near the upper ends of the grooves stubby colonies of *Pocillopora* dominate. In the surf zone *Pocillopora* gives way to *Acropora* (*A. pectinata, A. digitifera,* etc.). Other prominent coral genera here are *Astreopora, Porites, Millepora* and *Favites*.

The exposed windward reef tract is characterized by Type IB, which has a relatively high, prominent algal ridge cut frequently by grooves. The broad, rocky flat supports much less *Chama* and the *Microdictyon-Calcarina* association is less evident. Toward the outer slope *Acropora cuneata* occurs in shallow depressions, but even this species is not abundant. One is impressed with the very striking difference between the horizontal zonation of the windward reefs of Arno and those of Bikini Atoll. The corresponding reefs at Bikini are much wider and are characterized by broad outer and inner *Heliopora* zones which contained rather large subcircular masses of this blue alcyonarian in the form of micro-atolls (Ladd, Tracey, Wells and Emery, 1950).

The third seaward reef type (Type IIA) in which the grooves are weak and the margin is smoothly scalloped was found only in a small area at Ine Anchorage (Fig. 2). This area is distinctly zoned by corals, the dominant genera of which are shown in Figure 4. The inner portion of the reef flat is barren and exposed for a long period at every low tide. Extending seaward is a broad band thickly covered mostly with a pure stand of *Montipora gaimardi*, replaced here and there with patches of *Acropora hebes* or alcyoniid "soft corals" (*Lobularia krempfi*). A few micro-atolls of *Synaraea, Leptastrea* and *Porites* occur at random. During low tide in this zone the depth varies from exposed surfaces to shallow depressions as deep as 15 inches, and the water is undisturbed by swells or waves of translation. The seaward limit of this band is at about the inner extent of the ordinary low tide waves of translation. From this point to the reef margin *Acropora* dominated, especially species of the corymbose *A. pectinata* type. This is the zone of the richest variety of corals and associated animals on the reef flat and, unlike other seaward reef types, calcareous algae are relatively very unimportant. At least 33 genera of scleractinians and hydrozoan corals were collected in this zone, but *Acropora* accounts for about 90 per cent of all the colonies. On the steep slope over the reef edge and in the re-entrants along the edge an even greater variety of corals was found, numbering at least 90 species. Moreover, this area supported the most flourishing stand of corals, dominated by the majestic pedestal colonies of *A. reticulata* which began about 6 feet below low tide level, where the water was only slightly agitated by gentle swells, and extended down to about 60 feet, below which level *Porites* dominated the slope.

Description of the Study Areas

Seaward reef transect

The position of this transect is seen in Figure 2. The most prominent facies present along the transect have been described earlier in the description of the Type IIA reef present only at Ine Anchorage, and the dominant organisms are indicated in several text-figures depicting the zonal distribution of both physical factors and corals.

At the position of the transect the reef tract is about 450 feet broad, and is entirely covered at high water spring tides by about 4 feet of water. At mean low water it is uncovered to the boundary between quadrats 5 and 6, some 200 feet from the beach (Fig. 4). A few large, shallow tide pools are present in the barren rocky flat extending from the beach to quadrat 3, a distance of about 75 feet. Several boulders in one of these pools provide sheltering crevices in an otherwise highly exposed substrate. From quadrats 3 to 6 there are innumerable shal-

low basins and channels which hold water when the general level of the rock surface is exposed. Quadrats 7 to 9 always contain water above the average reef rock level and, in addition, contain many shallow to deep sandy-bottomed channels thickly lined with coral colonies. Quadrat 9 is bordered on one side by a narrow re-entrant channel. The reef margin, included in quadrat 10, is elevated slightly and exposed at ordinary low water. The ocean swells, however, continually sweep this low coral ridge so that it is never exposed for more than a few seconds at a time. Very few depressions or channels dissect this region and the coral heads on it are sparse, low and rather heavy. The seaward slope is precipitous, descending nearly vertically to about 35 feet, then continues downward at approximately a 70 degree angle with the water surface. Re-entrant channels are common and border both sides of quadrat 10.

Ine Anchorage is in the lee of Ine Island, thus the water over this transect was rarely influenced by the usual northeast trade winds. Wave action, particularly during low tides, was virtually absent because of the protection afforded by Stony Point to the east of the transect and because of the steep slope of the island to seaward in this location. Ocean swells from the south moved onto the reef at an incident angle because of the northwest-southwest reef margin at the Anchorage. At low tide they produced low, gentle waves of translation which flowed over the reef flat toward shore as far as quadrat 6. At high tide, particularly when the swells were extensive, waves broke just shoreward of the reef edge and strong waves of translation swept onto the beach, carrying small boulders and gravel to and fro across the inner barren zone and up the rather steep slope of the beach. During such periods considerable suspended material made the water turbid, but it settled out quickly during quiet periods.

Lagoon reef transect

Because of time limitations the very broad windward reef tract on the lagoon side of Ine Village was passed over in favor of a narrower reef tract at Makin Islet (Fig. 3). This transect was approximately 400 feet in length, running from the boulder and beachrock shore line to a depth of about 40 feet on the lagoonward slope. At mean low water approximately one-half of the reef flat was uncovered. Inshore the flat, rocky substrate was sprinkled with large and small pieces of beach conglomerate. These provided virtually the only seclusion for the intertidal fauna. Sandy bottom became the dominant substrate at about 100 feet from shore.

Scattered about the outer 200 feet were clustered coral heads which, from the point of deflection from the reef flat to the lagoonward slope,

were growing from the surface of giant, irregular columns of dead corals. These were replaced at approximately 25 feet in depth with a thicket of *Acropora formosa*, which continued downward beyond vision.

The extreme clarity of water here, coupled with the presence of sandy substrate, the surface layer of which was composed of exceedingly fine particles which remained suspended for hours after being disturbed, denoted an area infrequently disturbed by wind or wave. There is no elevated coral or algal ridge at the margin, differing in this respect from the broader windward lagoon reefs which are often subjected to winds and crashing waves. The delicate growth form of the corals add further testimony to the quiescent physical state along this transect.

Review of the Literature

Horizontal zonation of corals and other reef flat facies have been observed and described in greater or lesser detail by almost every geologist and biologist who has studied coral reefs, but few have given more than passing attention to it. The more important general studies of zonation on coral reefs are those of Wood-Jones (1912) on Cocos-Keeling Atoll, Tracey, *et al.*, (1948) and Ladd, *et al.*, (1950) on reefs of Bikini Atoll, northern Marshall Islands, and Wells (1951) at Arno Atoll in the southern Marshall Islands. Wood-Jones pointed out that on Cocos-Keeling Atoll various forms (not species) of corals had a fairly defined site of election; but in spite of this their distribution was capricious, there being no particular distinction between lagoon corals or sea reef corals. The facts that particular species might be found both on the lagoon and on the sea reefs, and that environmental modification of corals provided many growth forms of a single species led him to suggest that the distribution of atoll corals is a distribution of types, and *not of species* (italics his). Moreover, he recognized that the unfavorable conditions of crashing surf at the reef margin outweighed the assumed advantages of better aeration and more abundant food so that in this zone living coral reached its minimum of colonies as well as of species. At Cocos-Keeling the corals increased in number and variety toward the sheltered lagoon waters. A general survey of the reefs at Bikini by Tracey, *et al.*, resulted in a preliminary zonal analysis of the seaward reef and a classification of main seaward reef types based upon the characteristics of the marginal zone. Later more detailed analyses were presented by Ladd, *et al.*, whose consideration of the distribution of the coral fauna included the lagoon floor, coral knolls or patch reefs, the lagoon reef flat, the seaward reef flat and the seaward slope down to a depth of 800 feet. No suggestions concerning the relation between environmental factors and the distribution of corals were made. Coin-

cident with the detailed study of portions of the reefs of Arno reported herein, the broad aspects of zonation there were examined and reported upon by Wells.

The most significant detailed studies made on restricted areas of coral reefs are those by Mayor (1918, 1924) on the fringing reefs of Maër Island, on the northern end of the Great Barrier Reef, and in Tutuila, Samoa; that by Baker (1925) on a fringing reef of Gaua Island in the New Hebrides; and that by Manton (1935) on reefs of the Low Isles, a Low Wooded Island type just north of Trinity Opening in the Great Barrier Reef.

The extensive studies at Maër Island and on Aua reef, Tutuila, by Mayor have provided a wealth of detailed ecological data on the distribution of corals. Unlike Wood-Jones, Mayor concluded that each species of coral thrives best at some definite distance from shore and found but three genera (*Porites, Pocillopora* and *Goniastrea*) which extended clear across the reef flat at Maër, and only one so distributed at Aua (*Pocillopora*). Each of the common corals was found to attain a well-defined maximum in number of heads at some definite distance from shore and declined in frequency both shoreward and seaward. In general he found the maximum number of coral heads some distance behind the edge of the reef in relatively quiet water, but the greatest variety of corals was found closer to the outer edge. The distribution of corals on both reefs was somewhat similar, with zonation well established. However, most of the colonies at Maër consisted of but four genera (*Porites, Pocillopora, Acropora* and *Seriatopora*), while at Aua reef *Psammocora* replaced *Seriatopora*. Mayor concluded that the factors chiefly responsible for the mode of distribution of the various species of corals at Maër Island were, in the order of their importance, (1) temperature, (2) silt, (3) mechanical effects of moving water, and (4) the struggle for existence between the various species of corals. In the New Hebrides, coral growth appeared most abundant near shore where *Montipora ramosa* and *Goniopora* were dominant, with *Montipora* covering about 85 per cent of the reef surface. Farther out other genera (*Porites, Acropora*) dominated certain areas up to about one-fourth mile from shore, and from that point seaward coral growth was comparatively sparse. The traverses across reefs of the Low Isles by Manton cannot be compared with those previously outlined because of the rather different form and position of the reef tracts examined. Coral growth here was most abundant in the deeper part of the Moat and on the seaward slopes, with zonation evident only on the latter. Factors considered to be most influential in the distribution of corals on Low Isles were (1) level of the substratum in relation to that of low

water and to the maximum duration of low tides, (2) the ranges of temperature and salinity of the water, and (3) the nature and amount of the sediments which are deposited and which may lie undisturbed on rocks and corals.

All those investigating zonation of corals on reefs concur that no one environmental factor is solely responsible, but there is no concordance concerning the ranking in importance of those most influential. The more important studies of the deleterious effect of silt and the varying ability of different corals to remove it from the surface of the polyps were made by Wood-Jones (1912), Mayor (1918, 1924) Edmondson (1928), Manton (1935), Umbgrove (1947) and Wells (1951). In general, corals confined to the seaward areas are less able to withstand suspended silt or a covering of silt than those characteristic of the mid-reef flat and inshore areas. Moreover, the differences observed by contrasting the coral species and their distributional patterns on seaward vs. lagoonward reefs are believed to result mostly from finely particulate silt in the lagoon and larger, sandy sediments on the more turbulent seaward reefs.

Exposure to air is an inhibiting factor to virtually all corals, yet a certain few exceptional species are ubiquitous on the shoreward portions of reef tracts which are elevated slightly above the level of mean low water. *Montipora ramosa* appears to be the dominant type in exposed inshore areas in the New Hebrides, Low Isles, Maër Island, and in the East Indies; indeed, it thrives so well in this location that it has given rise to the designation *"ramosa* facies" by Umbgrove (1947). At Arno Atoll *M. gaimardi* occupies a similar location (Wells, 1951). A few species of corymbose *Acropora*, particularly *A. hebes*, are also common constituents of the fauna at Arno in areas exposed for brief periods each day. Vaughan (1916) found that the ability of corals to withstand exposure to air is a function of the porosity of the skeleton, the species with the more porous surviving longer than those with the denser skeletons. Thus porous types, such as *Montipora* and *Acropora*, with their bases in water are able to survive because the water is elevated through the skeleton by capillarity. Experiments by both Mayor (1918) and Edmondson (1928) on compact and cavernated types exposed to direct sunlight, some with and some without their bases immersed in water, corroborated Vaughan's earlier conclusions.

Most investigators consider the temperature gradient across a reef to influence the distribution of corals. Solar radiation causes greater temperature fluctuations in shallow inshore waters than it does at the reef edge, thus excessively high temperatures during certain days of the year could restrict inshore waters to a few hardy species (Mayor, 1918,

1924; Wells, 1951). Some experimental evidence presented by Mayor (1918) indicates that certain genera restricted to the edge of the reef were killed by temperatures not exceeding 37° C., yet inshore types thrived until water temperatures rose well above 37° C. On Hawaiian reefs Edmondson (1928) was unable to secure such clear-cut correlation between temperature tolerance and the position of corals on the reef.

Generally speaking dilution of sea water has not been considered one of the critical limiting factors in the distribution of corals except for inshore areas and for periods when low tides, torrential rains and calm seas combine to kill corals over considerable areas. Vaughan (1916), Mayor (1918) and Edmondson (1928) found that most corals were not adversely affected in water having a salinity of 75 per cent of normal sea water, but that greater dilutions would kill corals within a few hours. Mayor could find neither a higher concentration of sea water at low tide due to evaporation nor a dilution of sea water due to an influx of spring water from the shore at Maër Island. This latter situation may have resulted because his visit coincided with the end of a long dry spell. Thus, the record to date would imply that fluctuations in salinity are not of paramount importance in the zonation of corals except for those well inshore.

A factor long presumed to be significant in the distribution of corals, but one which has been neglected except for the work of Verwey (1931), is available oxygen. Working both on oxygen consumption of individual corals and of sections of reefs he concluded that the oxygen demands would necessitate considerable water motion, and where such movement is absent or slow reef growth may be depressed.

The record thus far gives us very little information as to what extent the distribution of corals may be influenced by competition between species for available space and food. Mayor (1918) suggests that in the surf zone where coral heads are small and not densely crowded, their struggle for existence is mainly with the sea as an adverse environment and not with one another; whereas in the less agitated water where the environment is favorable and the coral colonies are crowded, there is abundant evidence of a severe struggle between various forms for dominance, and the kinds that can survive are few. Manton (1935) recognizes the possibility of such an effect because the thickly growing heads of *Acropora* appear to discourage the establishment of other species among them.

Previous studies thus indicate that not only do several factors influence the distribution of corals on reefs but that these factors themselves are variable, exerting their limiting or inhibiting effects on different sections of the reef tract from the shore to the reef's edge. Factors

affecting inshore distribution, in order of rank, appear to be exposure, temperature and salinity; those affecting mid-reef areas are thought to be sedimentation and interspecific competition; while at the reef's edge the impact of waves seems predominant. Differences in the distribution of corals on windward and leeward reefs are attributed to sedimentation and the turbulence of water.

Acknowledgments

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Much of the value in this project emerged by cooperative assistance with colleagues in other fields. Thus to Dr. Donald W. Strasburg, who assisted in the marine studies, I owe a great debt of gratitude. Doak C. Cox, hydrologist; John W. Wells, geologist; and Leonard Mason, anthropologist, were most helpful in providing useful information. Without the services of James Milne and Konto, native interpreters, our work would have been far more difficult and less complete.

Methods

The seaward reef transect was a strip 50 feet wide from the shore to the slope over the reef margin. At intervals of 50 feet along the transect rock mounds were erected so that the area could be examined in quadrats 50 feet on a side. The lagoon transect was laid out similarly except that the quadrats were 100 feet on a side.

In each quadrat estimates were made of the amount of reef surface covered by the dominant corals, and a map was constructed showing their distribution. Specimens of each coral type in each quadrat were collected and prepared for taxonomic study.

Measurements of water temperature were taken in the center of each transect at both high and low tides at intervals throughout the summer period. Whenever extreme weather conditions prevailed sea reef and lagoon reef temperatures were taken so that data during extreme as well as normal weather conditions could be considered. All temperatures were taken at the level of coral growth by holding the

thermometer on the tops of coral heads and reading it underwater with the aid of a diving mask. Temperatures in quadrat 11 on the sea reef slope and in quadrat 4 on the lagoon reef slope were measured at a depth of 20 feet by diving down with the thermometer and holding onto coral heads for one minute, then reading the instruments before rising to the surface.

Water samples for chlorinity determinations were taken in the center of each transect at the same depth and time as the temperature readings were made. Chlorinity analyses were made in the field with a Wheatstone conductivity bridge which was calibrated against samples of sea water titrated with silver nitrate solution. Properly preserved samples were returned to Honolulu for accurate determination under better laboratory conditions, after which all field determinations were corrected.

Accurate determinations of levels of the sea reef platform, of the tops of coral heads and of the adjacent beach were made with an alidade; these levels, in turn, were related to the datum which was 0.0 on a staff attached to a portable tide gauge fixed on a re-entrant channel at the margin of the reef. Datum level was 4.1 feet above mean sea level, 1.9 feet above mean low water, and 1.9 feet below mean high water.

The tides were measured at the margins of both the lagoon and sea reef by portable, recording tide gauges supplied by the U. S. Coast and Geodetic Survey. From these brief records the U. S. Coast and Geodetic Survey computed the tidal relationships by referring all elevations to mean sea level in the ocean. The following data were computed:

	at ocean	*at lagoon*
Mean high water	1.90	1.93
Half tide level	0.00	–0.02
Mean sea level	0.00	——
Mean low water	–1.90	–1.97
Mean low water spring	–2.75	–2.82

Because climatic data taken for a period not exceeding two months is very inadequate to indicate changes even through a single season, the data are fragmentary. These, however, have been correlated with other scant but longer term records on the neighboring atoll of Majuro so that an indication of the hydrologic factors in the climate of Arno can be estimated. The mean monthly rainfall recorded from July through November at Ine Village was 17 inches, equivalent to an annual rainfall of over 200 inches. Daily rainfall during the period of observation ranged from nil to over 4.14 inches. Most of this rain came in sudden

intense rainstorms. No relation of rainstorm frequency to time of day was noted. The air temperature during the period of operation of the weather station ranged from 73° to 90° F. with a maximum daily range of 19 degrees and a common daily range of 10 to 12 degrees.

Because this paper emphasizes the relationship between salinity gradients on the reefs and ground-water, a detailed account of essential ground-water principles appears later in the section on salinity under "Factors Influencing Zonation." The hydrologist at Arno, Doak C. Cox, made detailed studies on the ground-water and found it to function as a typical Ghyben-Herzberg system. Ground-water characteristics were measured in various ways, including recording gauges, in land surface depressions, retting pits, taro pits and wells.

Descriptive Zonation of Corals

Only three major zones, dominated by two prominent genera, exist on the seaward reef transect. These are shown in vertical section along with Figures 4, 6, 7, 8 and 10. Rather stunted heads of *Montipora gaimardi* are first found in the seaward portion of quadrat 3, approximately 75 feet from the edge of the sand beach. In quadrat 4 the abundance of *M. gaimardi* heads increases greatly with a few scattered representatives of *Acropora hebes, A. pectinata* and *Porites lutea* growing in shallow pools. The area most completely covered by *M. gaimardi* occurs in quadrat 5, and the species gradually disappears seaward in quadrat 7. It is easily the dominant species in quadrats 3 to 6 covering 150 feet along the transect. *Acropora* gradually replaces *Montipora* about midway in quadrat 6 and thoroughly dominates the coral growth from this point to the reef margin, about 250 feet further. On the upper 20 feet of the steep reef slope *Pocillopora* is dominant. This merges below with a mixed zone reaching downward to about 35 feet in which no type is outstandingly conspicuous. From this depth down to about 55 feet the large pedestal colonies of *A. reticulata* flourish in the relatively calm water and clearly dominate the area. Below 55 feet these colonies give way rapidly to a rather thick coral cover in which *Porites lobata* is most common.

That the number of kinds or species of corals increases steadily toward the seaward edge of the reef at Arno is easily seen in Figure 4. Compared to coral distribution described in various other reef areas, this pattern of species density differs from that usually encountered. Ordinarily the greatest species density has been observed at the point where the surge dies out in ordinary weather (Mayor, 1918, 1924; Baker, 1925). This would correspond to quadrat 9 at Arno, which contains fewer than three-fourths the number of species found in quadrat 11 lo-

cated down the steep outer face of the reef platform. In another aspect of the distribution of corals, the Arno reef agrees with studies made elsewhere in that the fewest species occur inshore, but species density increases consistently seaward.

Quite a different picture is presented when considering population density along the transect. The *Montipora* zone, covering quadrats 3 to 6, had by far the greatest area covered by growing corals. Only scattered bare areas existed in this region where approximately 75 per cent of the reef flat supported a flourishing growth of *M. gaimardi.* This situation appears to be a replicate of that reported by Baker (1925) in the New Hebrides and by Mayor (1918) at Maër Island, where *M. ramosa* was the exceedingly abundant species exposed by moderately low tides near shore. No other area within the transect even approaches the inshore portion as regards the density of the stand. Heads of *Acropora* and associated genera comprise the next most dense thickets in quadrats 7 to 9 just at the inner edge of the normal surge zone where water is always present. Fewest heads appear in quadrat 10 which contains the elevated reef margin frequently subject to the impact of breaking waves.

The distribution of corals on the lagoon transect was very different from that of the seaward reef. No thick stands of corals occurred; instead, individual colonies or clusters of them arose from a sandy substrate underlain with coral boulders or, in deeper water toward the margin and down the slope, were attached to the sides and tops of massive columns of *Porites andrewsi.* Corals did not flourish on the inner half of the reef flat and were abundant only in quadrats 3 and 4. The margin of the reef marked the boundary between quadrats 3 and 4, so corals in quadrat 4 were characteristic of the lagoonward slope of the reef tract. On the inner half of quadrat 3 a mixed zone occurred in which no clearly dominant genera emerged. Rather, *Acropora, Stylophora, Astreopora,* and *Seriatopora* heads were present in about equal numbers. As the water deepened to approximately 4 feet at mean low water near the outer boundary of quadrat 3, the massive irregular columns of *P. andrewsi* clearly dominated the scene and very few colonies, aside from pedestal colonies of *Acropora* in the sand, grew from any other substrate. Heads of *Acropora* and *Astreopora* growing on dead portions of the *P. andrewsi* columns were dominant. Down the reef slope from 10 to 25 feet pedestal colonies of *A. reticulata* were very abundant, interspersed with loosely branched, erect colonies of *Hydnophora* and *A. formosa.* Below this depth thickets of *A. formosa* flourished and apparently extended across the entire floor of the lagoon.

Unlike the distribution of species across the sea reef the kinds of corals in the lagoon transect appeared to show no zonation within the

section of the reef which was not completely inhibiting to coral growth (Fig. 5). Species density on the lagoon reef was generally less than on the sea reef, due almost certainly to the comparative lack of suitable substrate for attachment of colonies. Nearly every coral boulder sufficiently large and stable to support a living colony was occupied. Population density, however, did vary, with the bulk of the individual colonies located in quadrat 3, just shoreward from the margin of the reef. This area also had a slightly greater number of species present.

It is apparent that corals on the sea reef flat and outer slope are zoned both as regards kinds and numbers of colonies, whereas those on the lagoon reef flat show virtually no zonation as regards kinds and but little zonation with respect to head density. However, zonation of kinds of species is evident on the lagoon reef slope.

Factors Influencing Zonation

Exposure

Relationships of levels of the reef rock platform, the top of coral heads and the gross aspects of zonation on the sea reef transect to the tidal periods and levels are shown in Figure 6. The entire reef platform, including the corals, is covered at mean sea level. When the tide is at this level or above swells rarely peak up and break at the reef margin, but instead sweep on shoreward to break first about quadrat 6. At mean high water the waves rarely break before reaching quadrat 2 or 3, consequently the heavy impact is spent either on the barren reef rock or on the sand beach. During periods of inclement weather breaking waves may occur at any place from the margin inward.

At mean low water the shoreward half of the reef from midway in quadrat 5 is exposed, thus for several hours each day this portion of the reef is without water except for that held in shallow depressions and pools. It will be noted in Figure 6 that from just behind the reef margin to about quadrat 6 the top two inches of most of the coral heads will be exposed. From this point to quadrat 3 entire coral heads will be exposed unless they are situated in depressions. The rather sharp break between the inshore *M. gaimardi* zone and the seaward *Acropora* zone also occurs at this point, thus implying that exposure is a limiting factor of considerable importance for types other than *Montipora*. *M. gaimardi*, like *ramosa*, is able to withstand long periods of exposure whereas *Acropora* is more severely limited in this respect, permitting *Montipora* to flourish in a thick, nearly pure stand shoreward from the inner extent of ordinary low tide waves of translation. Close inspection indicates that these *gaimardi* colonies actually have their

bases covered with 1 to 3 inches of water because they are located in very shallow depressions. Vaughan (1912) first suggested that cavernated forms like *Montipora* and *Acropora* could survive exposure to air and sun for long periods so long as their bases were in water, whereas compact corals were unable to withstand such exposure. Spongy forms, provided their bases are immersed in water, can draw up water by capillary attraction so that they retain their internal moisture even though exposed to the desiccating effects of the mid-day sun. Vaughan (1916) noted later in the Bahamas that species with porous skeletons also withstood greater desiccation than other types, and Mayor (1918) described a similar situation on Maër Island reefs. Mayor, and later Edmondson (1928), put this hypothesis to experimental test and fully corroborated the assumption made from observations in the field. The distribution of *M. gaimardi* at Arno may be interpreted as being influenced principally by the amount and duration of exposure to the direct rays of the sun. However, other factors, for example, temperature and salinity, also appear important in influencing this distributional pattern and certainly act in concordance with the tidal cycle.

Because mixed, semidiurnal tidal rhythms are characteristic on Arno shores, the distribution of many intertidal and subtidal species is undoubtedly influenced by "critical tide factors" as described for the Pacific Coast of the United States by Doty (1946). Although marigrams of Arno tidal features are not available over extensive periods, it is evident that successive high tides or successive low tides are often of appreciably different heights. As regards high tides, there is an alternation of higher high water with a lower high water, followed in turn by another higher high water, etc. Successive low tides show similar alternation. As might be expected the elevational aspects or variations of tide factor activity seem to be associated most frequently with the highest or lowest elevations of these four tides, and Doty (1950) has designated eight critical factors accordingly. These factors may, of course, be critical for a particular day, or for any period, depending upon the organisms and the modifying factors influencing tidal levels.

The true aspects of tide factor activity are associated, for corals at least, with effects on them of the duration of a single submergence or emergence at particular levels. For example, should the maximum height of lowest lower high water not exceed 0.5 feet then the corals shoreward from quadrat 6 would be exposed or have only their bases in very shallow water for a period as long as 17 hours, whereas corals below this level would be exposed or would be in very shallow water only for a maximum of about 8 hours. Along the western and eastern

coasts of the United States rather sharp breaks in the vertical distribution of sedentary intertidal organisms have been found to coincide with the predicted levels for these tide factors. Exposure (i. e., desiccation) alone has not been found so important a factor in zonation as was generally believed, but ancillary factors which can exert their greatest influence during periods of exposure, e. g., temperature elevation during clear sunny periods, rain or other sources of dilution, freezing, etc., have been suggested as most important (Doty, 1950). The extent to which various factors may act in accord with exposure will be discussed in subsequent sections of this paper.

A large number of micro-atolls occur in channels or depressions in quadrats 5 to 7. Most, if not all, of these have been formed by the killing and slight excavation of the center of colonies of *Porites* and *Synaraea* which grow up above mean low water. Despite the fact that the formation of micro-atolls is usually attributed to effects of sedimentation, influences of this sort have been unimportant in their formation at Arno. Rather, unfavorable environmental factors related in some way to periods of exposure have regulated the formation of these micro-atolls. Nothwithstanding the vigorous growth on the part of these compact corals, they are unable to elevate their colonies above the water level of the channels and pools. The edges, however, grow outward as thick as the depth of the pool. In many cases the colonies are 3 to 5 feet in diameter. This type of micro-atoll formation is precisely the same as that on the windward Bikini reefs (Ladd, *et al.*, 1950) and reefs in the Talaud Islands, East Indies (Kuenen, 1933).

No coral species in the lagoon occupied a comparable location to *M. gaimardi* on the sea reef. During spring lows the tops of large colonies of *Porites lutea* and *Pocillopora eydouxi* were exposed, but only for brief periods of time. Thus exposure to the direct rays of the sun appeared to be an inhibiting factor for species of corals occupying the lagoon reef.

Water movement

Water transport and turbulence varied greatly between the sea reef transect and that on the lagoon reef, even though both were rather protected. Water movement is evidently an important factor in zonation on the sea reef particularly as regards (1) the sharp break in species density noted between quadrats 6 and 7, (2) the large number of heads in quadrats 8 and 9, (3) the shape of the heads at the reef margin in quadrat 10, and (4) the distribution of the enormous pedestal colonies of *A. reticulata* on the steep seaward slope.

The boundary between quadrats 6 and 7 marks the inner extent of the waves of translation during normal low tides. Shoreward the

water is confined to pools and shallow depressions; seaward it is constantly being replenished with each succeeding wave. The favorable effects of moving water on corals such as continued high oxygen content (Verwey, 1931), replenished planktonic food supply, maintenance of rather high salinity values and the maintenance of temperature close to that of offshore waters, seem to be reflected in the greater number of kinds present. High population densities in quadrats 8 and 9 reflect all the favorable situations mentioned just previously, with an additional one—greater depth of water. The greatest turbulence occurs in quadrat 10 which includes the reef margin. Except for numerous channels the reef is elevated at the margin (see Fig. 6) thus subjecting coral heads both to brief exposure and to rather heavy wave action. The elevated areas are sparsely covered with a few hardy, heavy colonies of *Acropora* and *Pocillopora*, but the channels are thickly clothed with many species. The upper 15 feet of the outer slope, which is strongly influenced by the surge, is heavily clothed with a great variety of species, but below the surge zone the delicate pedestal colonies of *A. reticulata* grow vigorously down to a depth of about 60 feet.

Waves of translation are absent on the lagoon transect; the water there just rises and lowers quietly with the tide. There is no zone of breakers and no surge. Thus we would not expect sharp breaks in either species or population density. Moreover, since the surge is absent, pedestal colonies of *A. reticulata* begin at a depth of 5 feet near the margin of the reef and flourish downward to a depth of about 25 feet. Striking differences in water movement and turbulence on the two transects at Arno is therefore quite evident, with zonation on the sea reef influenced by them, whereas no distributional patterns related to these factors are apparent on the lagoon reef transect.

Silt

It has been stated elsewhere in this paper that the sea reef in the vicinity of the transect is virtually free from silt. During high tides when turbulence is greatest over the reef rather heavy particles of sand are suspended in the water, but these settle out very rapidly as the tide ebbs. Thus silt is a negligible factor in the distribution of corals and coral types on the sea reef transect.

A very fine, whitish silt covered the bottom on the lagoon reef transect, and it quickly rose in a cloud if disturbed. In normal weather the area was sufficiently protected so that suspended silt was largely absent. However, shifts in wind direction during storms would certainly stir this area making silt an inhibiting factor to those corals unable to withstand it. The bases of coral heads present were all dead

and the colonies themselves were rather widely scattered because sand and silt covered the bottom, leaving few boulders or dead coral heads exposed for attachment.

The most abundant heads in quadrat 3 belonged to the following genera, in order of abundance: *Seriatopora, Stylophora, Acropora, Astreopora, Goniastrea* and *Montipora.* Of these *Goniastrea* and *Acropora* have been previously shown to be very capable of withstanding considerable siltation (Mayor, 1918, 1924), but *Seriatopora* has therefore been believed to have very little resistance to sedimentation. None of the other genera have been examined experimentally as regards their resistance to silt. It is evident that much more experimental work must be done before silt as a factor in zonation of corals can be seriously considered. Especially is this so when many outstanding investigators have stated that silt is the most important factor in limiting or inhibiting coral growth (Wood-Jones, 1912; Manton, 1935; Umbgrove, 1947). Because of the conflicting reports on comparative experimental studies on the effects of silt on coral growth, it is impossible to make adequate analyses of the differences in kinds of corals found on the lagoon transect and on the sea reef transect. Clearly, corals on the sea reef are not zoned in response to silt as an environmental factor, yet neither are they zoned on the lagoon reef transect where much silt is present. If weather conditions did make the water turbid on the outer reef, it would be turbid entirely across the reef, thus obliterating any expressions of growth responses to differing amounts of silt in suspension. The fact that certain silt-resistant species are found on the lagoon reefs and not on the sea reefs does not *a priori* indicate that silt is the factor responsible.

Temperature

Variations in temperature along the sea reef transect were always greatest during low tides regardless of the time of their occurrence during the daylight hours (Fig. 7), but as would be expected, the greatest variation occurred during the afternoons. In every case the inshore water in pools in quadrat 2 was one to several degrees warmer than the air temperature taken at the same place and time. Actual temperature difference ranged from 1.4° to 11.0°F. Consequently, it is primarily solar radiation, not air temperature, which warms the water during the day. Unfortunately, no early morning temperatures were taken to determine whether or not the near shore water would be cooler than the air, thus it is impossible to detect the chief factor in cooling the water, although Mayor (1918) has suggested that radiation from the surface of the water is more important than the convection of heat

by the cooling air. In any event, no temperature records taken after 10 a.m. showed inshore water cooler than the air at that time.

During July and August it is evident that the ocean water 20 feet down the seaward reef slope ranges through only 0.5°F., but the range widens substantially during low tides towards the shore, reaching a maximum during our study of 12.3°F. in quadrat 2. This inshore range is only half as great as that found by Mayor at Maër Island where the surface sea water outside the reef ranged through 3.5°C. in October, indicating that the waters influenced by solar radiation on that broad reef flat affected the adjacent ocean waters far more than is true at Arno.

In addition to fluctuation in temperature across the reef flat at Arno, Wells (1951) discovered fluctuations along the reef at a particular distance paralleling the shore. Thus there are wedges of warm and cool water along the reef at low tide. The cool wedges were always found opposite re-entrant channels into which the cooler ocean water surged from outside the reef, whereas the warm wedges were always opposite the salients, characteristic of the transect area.

At high tide, regardless of the time of day, the temperature of the water over the sea reef flat is very consistent, and ranges only slightly above that of offshore waters (Fig. 8). The maximum range observed in quadrat 2 was 4°F. The rather consistent temperature from the reef margin inward to quadrat 5 is the result of strong waves of translation sweeping the cool ocean water shoreward. In quadrats 2 to 5 the warm water which was characteristic of the previous low tide still exerts a warming influence as it mixes with the sea water sweeping into it.

In the lagoon reef transect the range in temperature at low tide was approximately 5°F., far less than on the sea reef (Fig. 9). Moreover, the temperature of the shallow, rather still water in quadrat 2 was precisely the same as the air temperature of the time, 1°F. more in one instance and 1°F. less in the other.

It is clearly evident that if extremes of temperature influence the zonation of corals at Arno, they must act during periods of low water during daylight hours. Certainly the small range of variability during high tides on the sea reef would have no limiting effect on the growth or distribution of corals. On the lagoon reef there is no variation during high tides and at low tide the sharpest break in the temperature curve occurs about 250 feet from shore at the point where corals cease to flourish and only sand and reef rock occur shoreward. I do not ascribe the absence of corals shoreward from this 250-foot mark to

high temperatures, but rather to the fact that only 1 or 2 inches of water cover the area inshore which would make exposure an inhibiting factor.

No particularly sharp breaks in temperature occur across the sea reef at low tide, so the gross zonal limits of kinds of corals cannot be associated directly with the presence of periods of unfavorable or favorable temperature. Instead there appears to be a limiting influence exerted by temperature, principally felt inshore, but operative probably as far seaward as quadrat 10. Certainly the wide range in temperature in quadrats 2 and 3 would be deleterious to coral growth, but we have already noted that several other factors may be just as deleterious or more so. Temperature variation, then, at low tide would seem to be a contributing factor in limiting the growth of certain corals, but it does not appear inhibiting because no sharp breaks in the zonation of corals coincide with the typical temperature curves. There is no justification for attributing zonation of corals at Arno to temperature differences as has been done by Mayor for the very broad reefs of Maër Island and of Samoa. Neither can temperature be considered one of the most important factors in zonation as was done at Bikini by Ladd, *et al.*, (1950). Rather it must be concluded that the vicissitude of temperature fluctuations shoreward makes the inshore area less favorable for coral growth than the region toward the margin where temperature conditions approach a rather steady state of little change. Evidence supporting this may be found in the distribution of such resistant forms as *Porites lutea*, which is a common inshore form but is not found near the reef margin. Experimental evidence purporting to permit the interpretation of species distribution from the degree of resistance of corals to increasing temperature is not convincing; indeed, Edmondson (1928) found that the ability of a species to endure high temperatures does not always indicate it to be a near shore form, and that resistance to temperature is not well correlated with the position of the colonies on a reef.

Salinity

Contrary to the situation at Maër Island where Mayor found virtually no dilution of waters over the reef flat either at various times of the day or states of the tide, the range of chlorinity along the sea reef transect during low tides at Arno was rather great, varying from as low as 64 per cent as concentrated as ocean water to 85 per cent (Fig. 10). A rather steady increase in chlorinity is evident from inshore waters to the reef margin where the water never varied more than 1.5 parts per thousand regardless of the tide. At no place is

there a sharp discontinuity. During high tides the chlorinity was almost invariable over the entire width of the reef tract because the waves of translation reached the beach.

On the lagoon transect virtually no chlorinity gradient exists (Fig. 11). On one occasion an inshore sample was measured which was only 90 per cent as concentrated as sea water, but in all other instances no variation was noted, state of tide and time of day notwithstanding.

Because very few measurements of chlorinity gradients across reef tracts have been made, and these indicated no appreciable dilution of reef waters, and because experimental results on subjecting corals to various dilutions of sea water suggest that corals might live indefinitely and without harm if the ocean salinity were reduced to 80 per cent of normal sea water (Vaughan, 1916), the factor of dilution has been more or less dismissed as insignificant in influencing the distribution of corals except when torrential rains may coincide with low water (Mayor, 1924). The same evidence upon which the foregoing conclusions were based may be coupled with what is generally known about "critical tide factors," as discussed previously in the section on exposure, to question seriously the previous concepts of dilution as a negligible factor in the growth and distribution of corals. Such data as that presented by Mayor for corals at Maër Island where only half of the reef corals could survive 11½ hours of 50 per cent dilution and only 3 could withstand this dilution for 24 hours; and that of Vaughan (1916) and of Edmondson (1928) for Tortugas corals and Hawaiian corals respectively in which they found a dilution to 75 per cent sea water not overtly injurious, but greater dilutions injurious, over extended periods indicate that dilution does have a depressing effect, although considerable variability in resisting dilution is evident among certain corals. It follows that, coincident with periods of low water, dilution and high temperatures can act in concert to limit the growth of some corals and inhibit other less hardy forms.

Because Arno Atoll is rather typical of other atolls of the same latitudes as regards geological composition and climate, many reefs must exhibit the same variable salinity pattern as found here. In checking possible reasons for the lowered salinity across the seaward reef at low tide, rainfall and tidal status appeared to be only incidental. For example in quadrat 2, during low water on the days when the samples were collected, the chlorinity was 12.8 parts per thousand, the tide was 0.5 and 1.6 inches of rain had fallen the previous night. When the chlorinity was 17.0, the tide was 0.6 and 3 inches of rain had fallen the previous two days. During these periods of great fluctua-

tion in chlorinity on the sea reef the chlorinity on the lagoon reef remained consistently high. The lack of fluctuation on the lagoon reef and the consistent dilution on the sea reef regardless of rainfall eliminated rainfall as a significant factor directly related to the dilution of reef waters. The only remaining source of fresh water on an atoll is ground-water. The author is indebted to Mr. Doak C. Cox, hydrologist, for clarifying the fundamentals regarding ground-water on an island such as Ine. Because this ground-water is the primary source of dilution of the reef waters, and because its principles are little known to biologists, its basic details are reviewed herewith.

Of prime importance to the accumulation and dissipation of ground-water on Arno are the geological characteristics of the atoll. Wells (1951) has described in detail the geological features of Arno which are significant in considering this subject. The islands on an atoll are mere heaps of sand and boulders on the reef platform or the top of the reef which gives shape to the atoll. Their position and the character of the materials within them are determined by shape, size and exposure of the sections of the reef on which they develop. Surmounting the beaches on the ocean sides of the island is a boulder rampart. The ocean beaches themselves contain many boulders interspersed with very little fine sand. The lagoon beaches, on the other hand, are composed primarily of sand, much of it exceedingly fine. Because the islands develop by accretions on both shores, the materials comprising the beaches on the two shores should be representative of the materials composing the islands as a whole. Thus, the permeability of the coarse ocean beach sediments should be much higher than that of the fine lagoon-beach sediments. Whether these surface conditions are typical of the underlying materials depends on whether the present environments are typical of those which prevailed while the reefs were constructed. Since we must assume that the present and past environments are similar, there is a strong possibility for the development of asymmetry in the permeability of the island.

Rain falling on the surface of an island such as Ine is in part absorbed into the ground where it percolates downward and accumulates at the surface of the salt water at sea level. The porous island rock, partly submerged in ocean water, though it does not absolutely confine the water that saturates it, provides a restriction of flow that may reduce mixing sufficiently so that if fresh water is introduced into it, an integral body of fresh water will be maintained, floating on the denser sea water in the rock and displacing it so that approximately every unit of fresh water above the ocean water level will be supported

by 40 units below. This hydrostatic relationship is known as a Ghyben-Herzberg system (Wentworth, 1947; Cox, 1951).

It has been established that the amount of ground-water flowing through a sand of given cross section will be proportional to the hydraulic gradient, and that with the same hydraulic gradient in the same system but with sands of different sizes and degrees of compaction, the amount of water flowing through a given cross section will be proportional to the permeability of the sand.

On islands such as Ine the bulk of the water lost from the lens must flow to its margins at the shores, which flow can be induced only by a hydraulic gradient towards the shores. By the Ghyben-Herzberg law the higher heads inland must be balanced by greater depths of fresh water inland. If permeability were constant and the rainfall uniform all the excess rainfall must flow through the shore edges of the fresh-water body. Near shore the head is low and the thickness of fresh water small, thus this must be balanced by a high shoreward hydraulic gradient, and the rapid increase of both head and depth of fresh water inland. The fresh-water body, therefore, has a lenticular shape with a water table that is convex upward, and with a lower, salt water contact that is similar but exaggeratedly convex downward. Because sea level, which controls the position of the lens, has tides, the loss of water from the lens is controlled by a variable base, making the hydraulic gradients throughout the lens and the rate of loss variable.

Hydrologic studies have shown that Ine Island, at the site of the sea reef transect, has greater permeability on the ocean side than on the lagoon side as evidenced by the relatively low head inland from the ocean shore and the relatively high head inland from the lagoon shore (Cox, *op. cit.*). Differences in permeability were also indicated by the greater damping and lag of the tides as they move in from the lagoon shore than as they move in from the ocean shore. The water table gradients on the two sides of Ine Island indicate an effective permeability ten times, or more, greater in the zone occupied by the lens on the ocean side than on the lagoon side. A diagrammatic cross section of the Ghyben-Herzberg system at Ine Village as it is presumed to exist is shown in Figure 12.

Because the maximum head of the lens at Ine is approximately 1 foot above mean sea level, the action of the tides induces profound changes in the outflow onto the reef. The mean high tide level here is nearly three feet higher than the maximum head of the fresh water, thus the sea water effectively damps the outflow onto the reef, and, in fact, results in a reversal of slope of the hydraulic gradient. At

low tide, on the other hand, lens water flows rapidly onto the reef platform and dilutes the sea water there.

If the permeability of the sediments were the same on both sides of the island the lens would be more symmetrical and the inshore waters on the lagoon reef would become diluted by the fresh-water outflow. This does not occur because of the impermeability of the fine sand characteristic of the lagoon side of the island and reef platform. The asymmetry in dilution on the two sides of the island is thus explained, and the basic principle almost certainly holds for other island areas. Variations will appear, of course, depending upon rainfall, permeability, size of island, tidal amplitude, etc.

These data indicate that lowered salinity is probably a usual occurrence during low tides across seaward reefs, and that in areas of mixed, semidiurmal tides this dilution may exist for as long as 17 to 19 hours inshore. The alteration in the metabolism of corals under these circumstances, particularly when coupled with exposure and high temperatures, certainly influences their growth unfavorably. Now and then extreme conditions occur in certain environmental factors which impart totally unfavorable situations for corals. For example, prior to the arrival of our instruments for determining chlorinity at Ine a very low, low tide was followed by a rather low, high tide, and this in turn was followed by a moderately low, low tide. During this period a torrential rain fell continuously, which, coupled with the ordinary outflow of fresh water from the lens, must have reduced the water from quadrat 6 shoreward to a salinity well below 50 per cent of normal sea water. The following morning the top three inches of the *M. gaimardi* heads had turned a light brownish color and by evening they had bleached white. That part of the colonies which had been subjected to the greatest exposure and dilution had been killed, indicating that a single day's exposure to a very unfavorable environment could be inhibiting to the hardiest of corals. It is not surprising then that the usual periods of exposure to diluted sea water during normal low tides would limit the growth of less hardy species on the shoreward portion of the reef flat.

Discussion

Of the various factors known to limit or inhibit coral growth, it appears that sharp changes or discontinuity in species distribution are influenced principally by exposure, and that critical tide factors, particularly at the level of "lowest lower high water," may be very important in the near exclusion of corals other than *M. gaimardi* from quadrats 3 to 5. The absence of any coral species on the inner, ex-

posed slopes of the lagoon reef indicates that exposure among lagoon corals at Arno Atoll is completely inhibitive.

As important as exposure is in itself, desiccation appears to be a rather negligible factor for *M. gaimardi*, because the dense clusters of heads are invariably in shallow depressions where trapped water is available to rise by capillarity through the cavernous skeletal structure. If desiccation were the only factor involved then other spongy forms such as *Acropora* might also invade the shoreward reef flat. The differential distribution of corals inshore coupled with the development of micro-atolls implies that two or more factors are effective in regulating distribution. It has been mentioned that during periods of low water both solar insolation and dilution contribute to a widely fluctuating environment wherein only the hardiest forms find favorable existence. Seaward the environmental fluctuations are minimized until a very consistent environment is attained on the outer seaward reef slope just below the margin. The distribution of species across the reef at Ine Anchorage is an interesting one because, unlike population density which represents an absolute value and is based primarily on the amount of food available, species density is a relative value and depends mostly upon the selective action of the environment. Under optimum conditions for existence the species density should usually be very great, but it would decrease as the environmental exigencies increase. The distribution of species along the seaward reef transect at Ine bears out this hypothesis. Wide fluctuations inshore in exposure, temperature and salinity, all of which are intimately associated and mutually dependent, become progressively less variable toward the reef margin. Increase in species density appears directly correlated with a decrease in environmental variability. Except for exposure, this decrease in environmental variability is a gradual one, and the data would indicate that the effect on coral growth of such fluctuations is limiting, rather than inhibiting, and that the distribution of kinds of corals is regulated by their individual ecological valence for these variable environmental factors. The lack of variable environmental factors along the lagoon reef transect, except for exposure, results in a rather even distribution of kinds of corals so long as the water covers them at low tide. This is precisely the type of distribution one would expect.

These data indicate that on the sea and lagoon reef transects at Arno Atoll, exposure is probably the most important single factor in the distribution of kinds of coral, and is almost singly responsible for the rather sharp division between the dense inshore stand of *M. gaimardi* and the other corals seaward of it. Coincident with low water,

high temperatures from solar radiation and lowered salinities from ground-water outflow mutually induce the unfavorable environment for coral growth, which becomes less limiting toward the margin of the reef. The gradual decrease in species density from the margin shoreward may be attributed to their varying tolerances to these unfavorable environmental factors. The greatest species density, found on the outer reef slope just over the margin in quadrat 11, is indicative of very favorable environmental conditions which show virtually no fluctuation daily, seasonally or annually. Silt deposition is unimportant on the sea reef, and water movement here influences primarily the form of the coral head rather than being inhibitive to particular species.

The factor of competition among corals on the sea reef transect looms up only in quadrats 6 to 9. Shoreward of quadrat 6 and in the wave impact area of the reef margin in quadrat 10, the vicissitudes of environment appear more important in the welfare of species than neighboring coral heads. In quadrats 6 and 7 *Acropora* gradually replaces *M. gaimardi* as the dominant form. Both types are corymbose and grow in thick clusters. Shoreward of quadrat 6 the environment apparently limits the vigorous growth of *Acropora* so that *Montipora* tends to crowd it out. However, if conditions are favorable for vigorous growth of *Acropora* it becomes very abundant and gives other species very little space for developing colonies. Thus, from quadrat 7 to the reef margin *Acropora* easily dominates the kinds present. Other types are located mostly in channels or depressions, and many are encrusting forms which grow in small patches on the bases of erect species. At the reef margin the heads are small and compact, leaving room for many other species capable of withstanding the impact of waves to gain a foothold.

On the lagoon transect competition between corals was accentuated even though they were not closely packed, because suitable substrate for attachment and growth was at a premium. Here erect species attached to coral boulders or dead massive heads were in severe competition with encrusting types which thickly clothed all boulders, dead columnar heads and even the bases of the live, erect types. Since virtually no adversities in environment had to be met, except for an occasional period of heavy silt suspension, the competition was mainly between coral and coral.

From the standpoint of understanding the total ecology and productivity of coral reefs, too much emphasis cannot be placed on the distribution of corals because they provide the key to the distribution and abundance of virtually every other animal and many of the plants pre-

sent. Reefs in which corals rather than nullipores play the dominant role, such as those described at Arno, form communities in which the coral heads provide excellent protection and concealment for many species. The cavernous nature of the massive corals make near perfect hollows for the retreat of many fish and invertebrates, and the relatively soft consistency of the limy skeletons permits the existence of a multitude of burrowing forms. Thus, the entire assemblage of animals surrounding a coral reef will be concentrated on and in coral heads, and life abounds there in the midst of an otherwise impoverished area.

Summary

1. This study was undertaken to assess more precisely the factors responsible for the horizontal zonation of corals on the sea and lagoon reefs of Arno Atoll, Marshall Islands. Only corals are used as indicator species.

2. A transect 50 feet wide was marked out across the seaward reef tract. It was marked off linearly at 50-foot intervals to form quadrats 50 feet on a side. On these transects periodic measurements were made of chlorinity and temperature. Levels of the reef flat and of the tops of coral heads were measured in relation to a datum level. Collections of corals were made in each quadrat for taxonomic determinations and the head density was estimated for the most prominent types.

3. On the transect across the sea reef tract two clearly-defined zones of coral growth are evident between the shore and the margin. *Montipora gaimardi* thickly covers the inner portion of the reef from the beginning of coral growth to a point 200 feet from shore. Seaward to the reef margin at 450 feet *Acropora* easily dominates. Just over the reef margin *Pocillopora* is most prominent on the upper 20 feet of precipitous seaward slope, and below that *A. reticulata* and *Porites lobata* become most common. The number of species of corals increases consistently seaward and the outer reef slope supports the greatest variety of kinds, although the densest population of coral heads occurs about 100 to 150 feet shoreward from the margin.

4. On the lagoon reef transect no clear-cut zonation of corals was apparent. No type comparable to *M. gaimardi* existed on the lagoon reef, and exposure evidently was completely inhibiting to all lagoon species. On the lagoonward slope *Porites andrewsi* dominated to a depth of 10–15 feet, followed by a belt of pedestal colonies of *A. reticulata*. Below this thickets of *A. formosa* were abundant and extended to the lagoon floor. Fewer species occurred on the lagoon reef than were present on the sea reef, and population density was greatest on the outer portion of the reef flat.

5. Of the various factors known to limit or inhibit coral growth, it appears that sharp changes or discontinuity in species distribution is influenced principally by exposure. The absence of any coral species on the inner, exposed slopes of the lagoon reef indicates that exposure among lagoon corals at Arno is completely inhibitive. Coincident with low water, high temperatures resulting from solar radiation and lowered salinities derived from ground-water outflow mutually induce an unfavorable environment for coral growth shoreward, but these factors become less limiting toward the margin of the reef because of their diminished variability until they gradually merge into the unvarying values characteristic of oceanic waters.

6. The gradual decrease in species density from the reef margin shoreward may be attributed to varying tolerances to unfavorable environmental factors, and the greatest species density found just over the margin was indicative of highly favorable environmental conditions.

7. Silt deposition is unimportant on the sea reef, and water movement there influences the form of the coral head rather than being inhibitive to any particular species.

8. The lack of variable environmental factors on the lagoon reef results in a rather even distribution of kinds of corals and very little or no zonation, except shoreward where exposure is inhibiting.

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ILLUSTRATIONS

FIG. 1.—Map of Arno Atoll showing reef types (see text for details).

FIG. 2.—Map showing the reef tracts on the seaward and lagoonward sides of Ine Island at Ine Village. The seaward reef transect is shown extending outward from Ine Village.

FIG 3.—Map showing the position of the lagoon reef transect at Makïn Islet on the southeast corner of Arno Atoll.

FIG. 4.—Variation in the number of coral species from the beach to the edge of the sea reef along the study transect at Ine Village, Arno Atoll. A profile along the transect is placed below the graph to show precise relationships between the number of coral species and varying distances from shore.

FIG. 5.—Variation in the number of coral species from the beach to the outer slope of the lagoon reef along the study transect at Makïn Islet, Arno Atoll. A profile along the transect is placed below the graph to show precise relationships between the number of coral species and varying distances from shore.

FIG. 6.—Vertical section or profile of the sea reef transect at Ine Village, Arno Atoll, showing the levels of the reef rock platform and the tops of the coral heads in relation to tidal levels. MHW denotes mean high water; MSL denotes mean sea level, etc.

FIG. 7.—Temperature variation on the seaward reef transect at Ine Village, Arno Atoll, during afternoon low tides. A profile view of the transect is placed below the graph to show relationships between temperature variation and distance from shore.

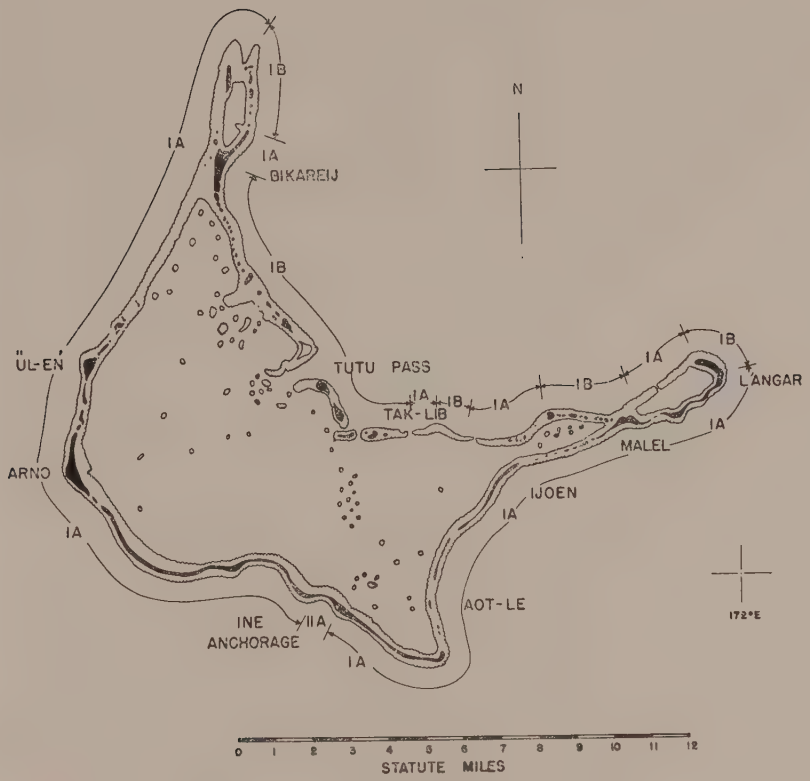
FIG. 8.—Temperature variation on the seaward reef transect at Ine Village, Arno Atoll, during afternoon high tides.

FIG. 9.—Temperature variation on the lagoon reef transect at Makïn Islet, Arno Atoll, during midday and afternoon low tides.

FIG. 10.—Chlorinity variation on the sea reef transect at Ine Village, Arno Atoll, during high and low tides. A profile view of the transect is placed below the graph to show relationships between variations in chlorinity and distance from shore.

FIG. 11.—Chlorinity variation on the lagoon reef transect at Makïn Islet, Arno Atoll, during high and low tides. A profile view of the transect is placed below the graph to show relationships between variations in chlorinity and distance from shore.

FIG. 12.—Cross section of Ine Island, Arno Atoll, showing estimated total depth and relationships of the Ghyben-Herzberg lens.


N
IB
IA
IA
BIKAREIJ
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ÜL-EN
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LANGAR
IA
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ARNO
IJOEN
IA
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172°E
INE
ANCHORAGE
IIA
IA
0 1 2 3 4 5 6 7 8 9 10 11 12
STATUTE MILES

FIGURE 1

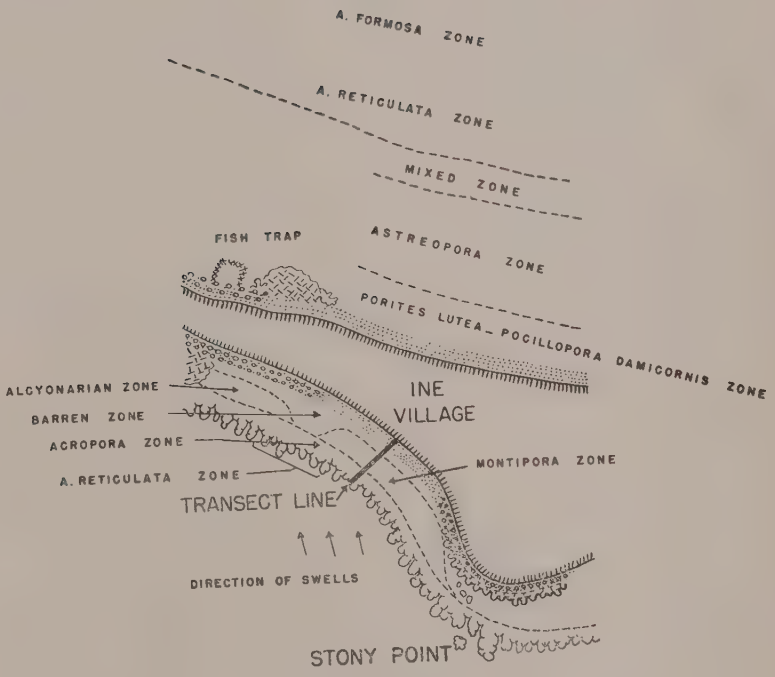

A. FORMOSA ZONE
A. RETICULATA ZONE
MIXED ZONE
FISH TRAP
ASTREOPORA ZONE
PORITES LUTEA
POCILLOPORA DAMICORNIS ZONE
ALCYONARIAN ZONE
BARREN ZONE
ACROPORA ZONE
A. RETICULATA ZONE
INE
VILLAGE
MONTIPORA ZONE
TRANSECT LINE
DIRECTION OF SWELLS
STONY POINT

FIGURE 2

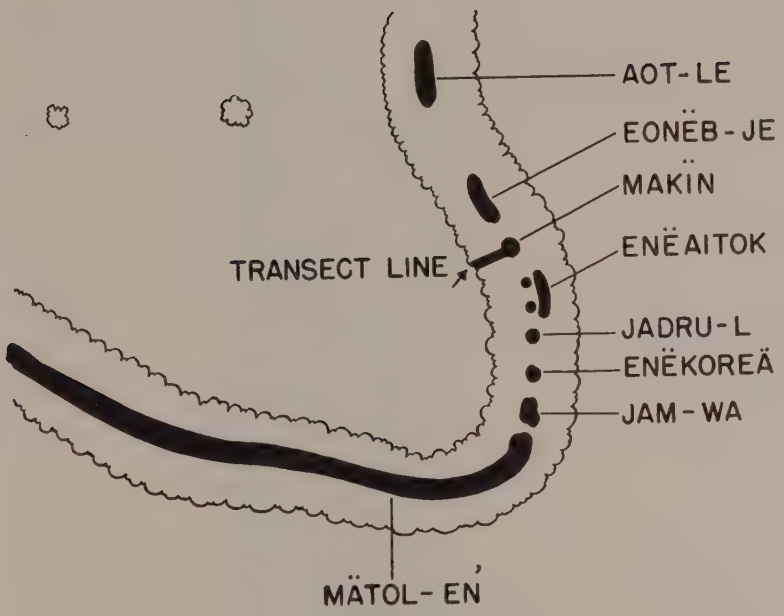

AOT-LE
EONËB-JE
MAKIN
ENËAITOK
TRANSECT LINE
JADRU-L
ENËKOREÄ
JAM-WA
MÄTOL-EN'

FIGURE 3

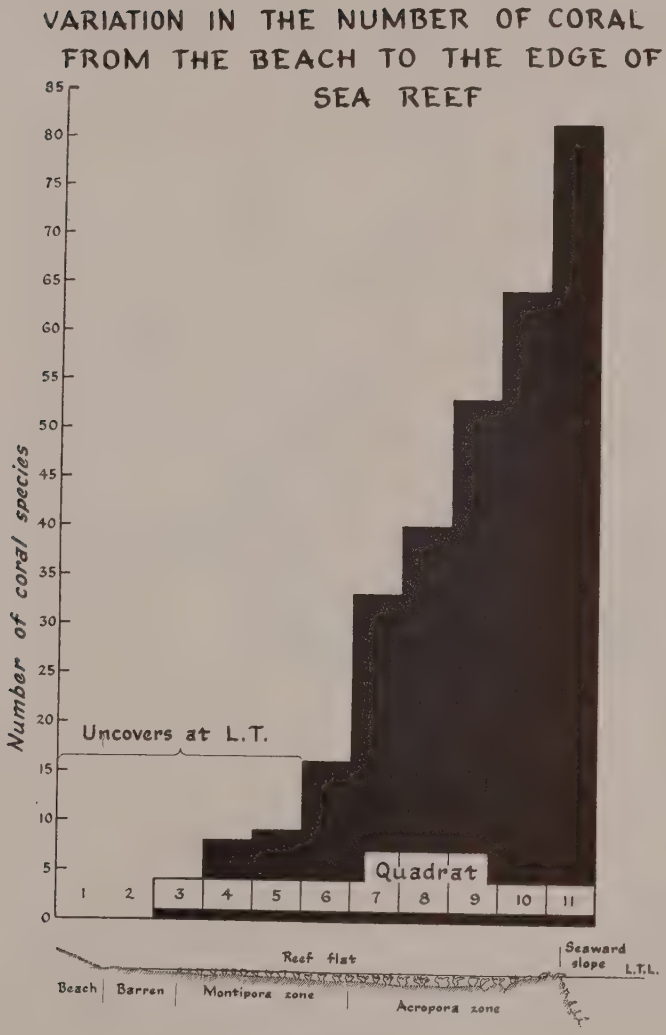

VARIATION IN THE NUMBER OF CORAL SPECIES FROM THE BEACH TO THE EDGE OF THE SEA REEF
Number of coral species
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Uncovers at L.T.
Quadrat
1
2
3
4
5
6
7
8
9
10
11
Reef flat
Seaward slope
L.T.L.
Beach
Barren
Montipora zone
Acropora zone

FIGURE 4

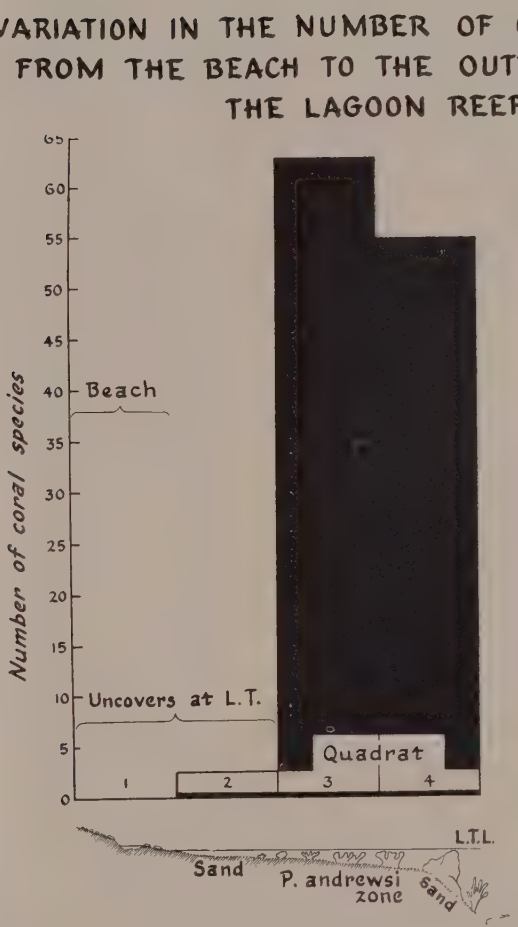

VARIATION IN THE NUMBER OF CORAL SPECIES FROM THE BEACH TO THE OUTER SLOPE OF THE LAGOON REEF
Number of coral species
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Beach
Uncovers at L.T.
Quadrat
1
2
3
4
L.T.L.
Sand
P. andrewsi zone
Sand

FIGURE 5

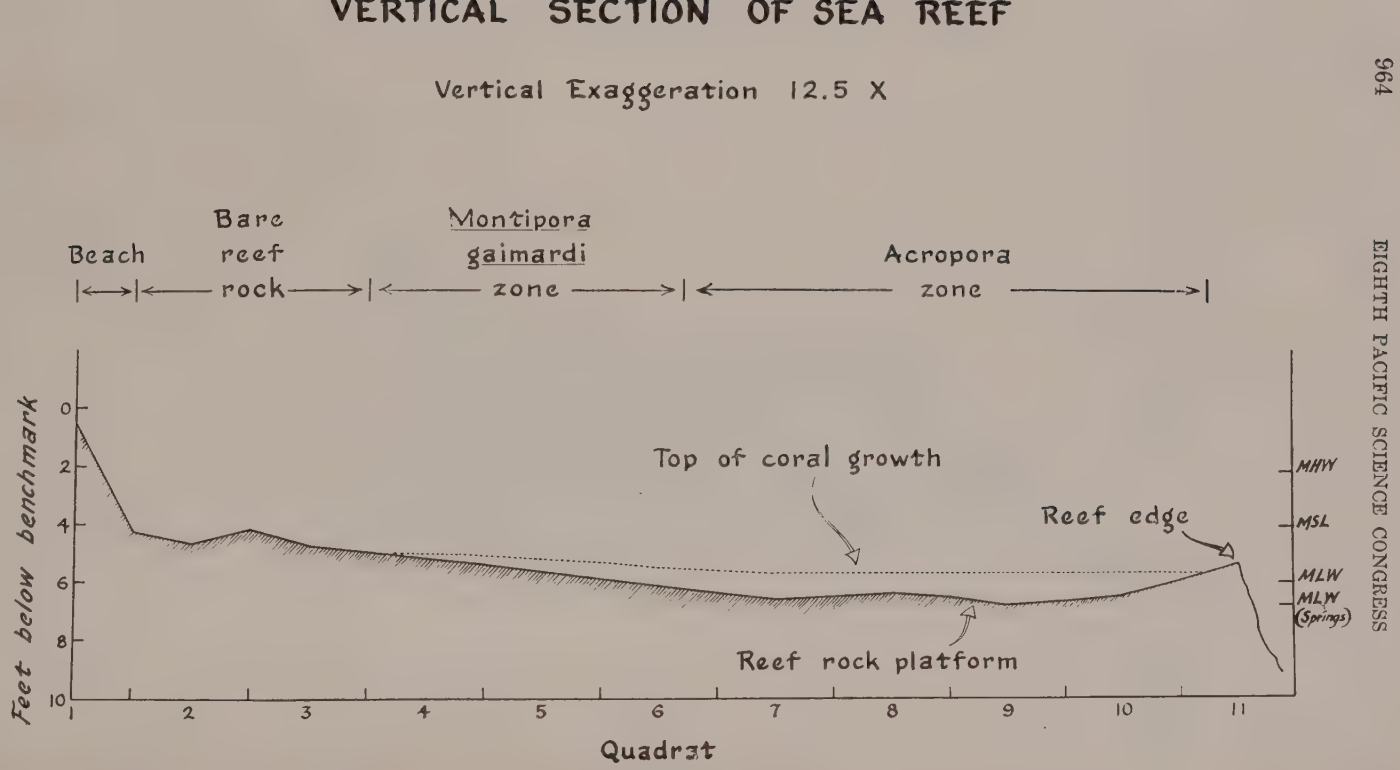

VERTICAL SECTION OF SEA REEF
Vertical Exaggeration 12.5 X
Beach
Bare reef rock
Montipora gaimardi zone
Acropora zone
Feet below benchmark
0
2
4
6
8
10
Top of coral growth
Reef edge
Reef rock platform
MHW
MSL
MLW
MLW (Springs)
1
2
3
4
5
6
7
8
9
10
11
Quadrat

FIGURE 6

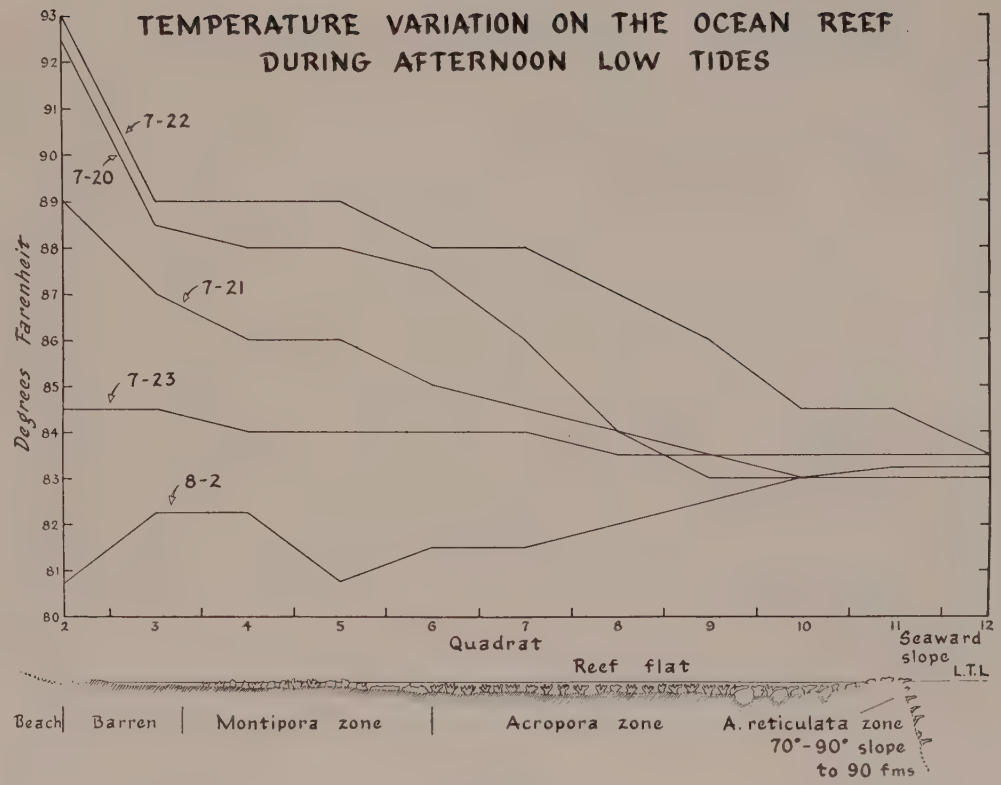

TEMPERATURE VARIATION ON THE OCEAN REEF
DURING AFTERNOON LOW TIDES
Degrees Farenheit
93
92
91
90
89
88
87
86
85
84
83
82
81
80
7-22
7-20
7-21
7-23
8-2
2
3
4
5
6
7
8
9
10
11
12
Quadrat
Reef flat
Seaward slope
L.T.L.
Beach
Barren
Montipora zone
Acropora zone
A. reticulata zone
70°-90° slope
to 90 fms

FIGURE 7

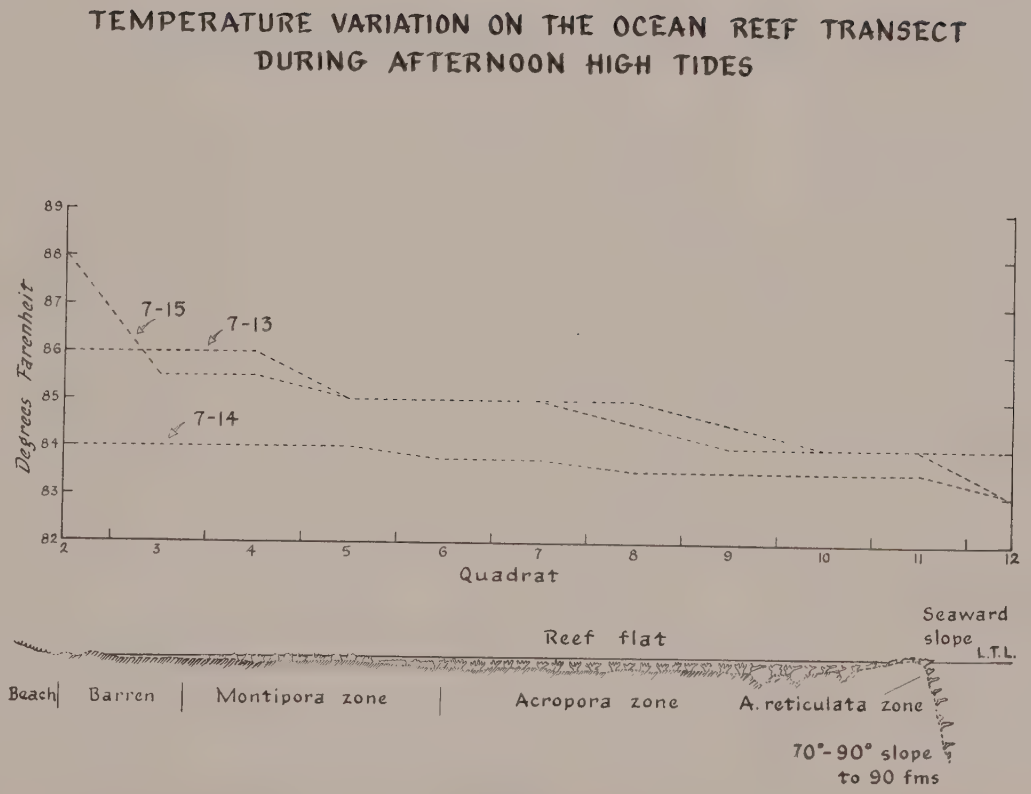

TEMPERATURE VARIATION ON THE OCEAN REEF TRANSECT
DURING AFTERNOON HIGH TIDES
Degrees Farenheit
89
88
87
86
85
84
83
82
7-15
7-13
7-14
2
3
4
5
6
7
8
9
10
11
12
Quadrat
Reef flat
Seaward slope
L.T.L.
Beach
Barren
Montipora zone
Acropora zone
A. reticulata zone
70°-90° slope to 90 fms

FIGURE 8

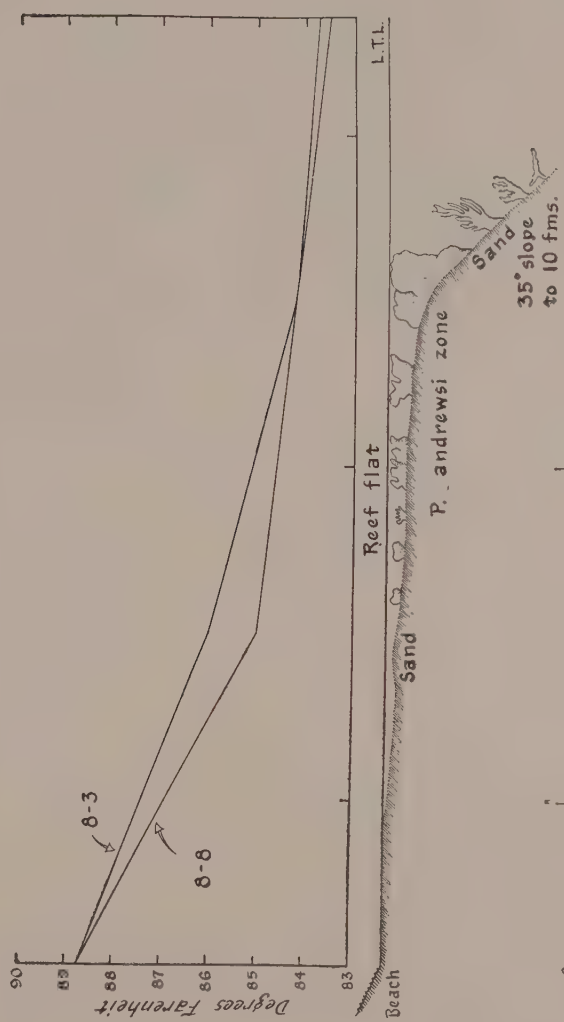

TEMPERATURE VARIATION ON THE LAGOON REEF
DURING MIDDAY AND AFTERNOON LOW TIDES
Degrees Farenheit
90
89
88
87
86
85
84
83
8-3
8-8
Reef flat
L.T.L.
Beach
Sand
P. andrewsi zone
Sand
35° slope
to 10 fms.
2
3
4
Quadrat

FIGURE 9

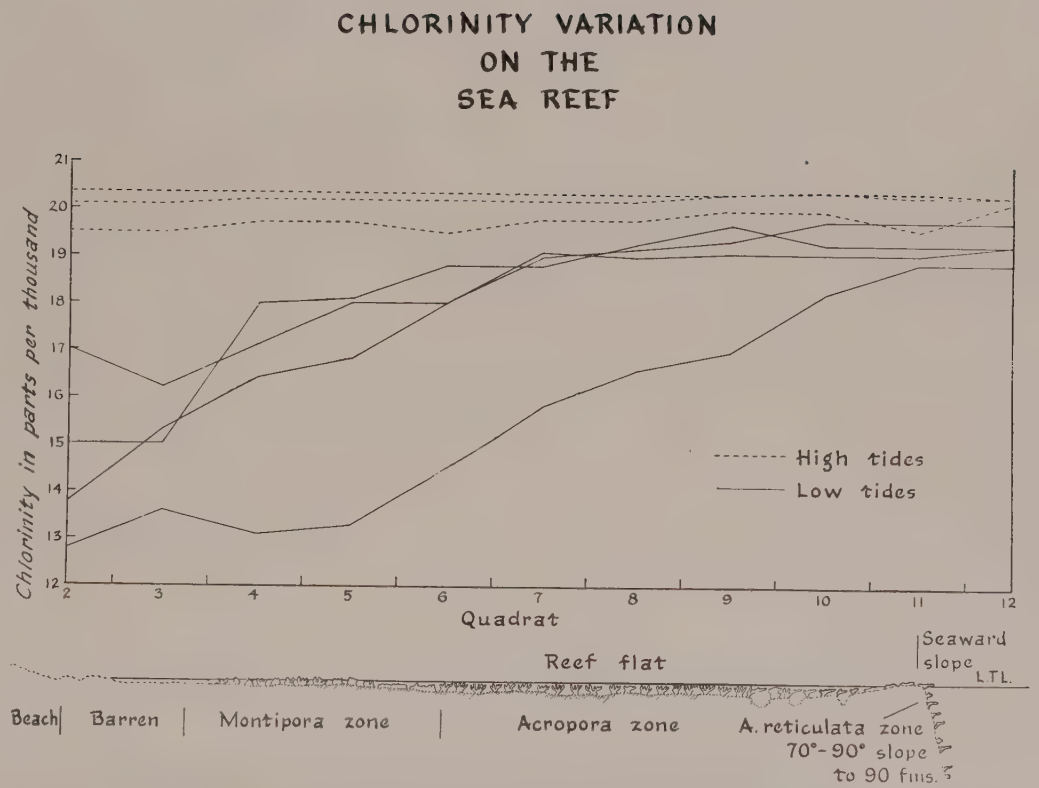

CHLORINITY VARIATION
ON THE
SEA REEF
Chlorinity in parts per thousand
21
20
19
18
17
16
15
14
13
12
2
3
4
5
6
7
8
9
10
11
12
Quadrat
High tides
Low tides
Seaward slope
L.T.L.
Reef flat
Beach
Barren
Montipora zone
Acropora zone
A. reticulata zone
70°-90° slope
to 90 fms.

FIGURE 10

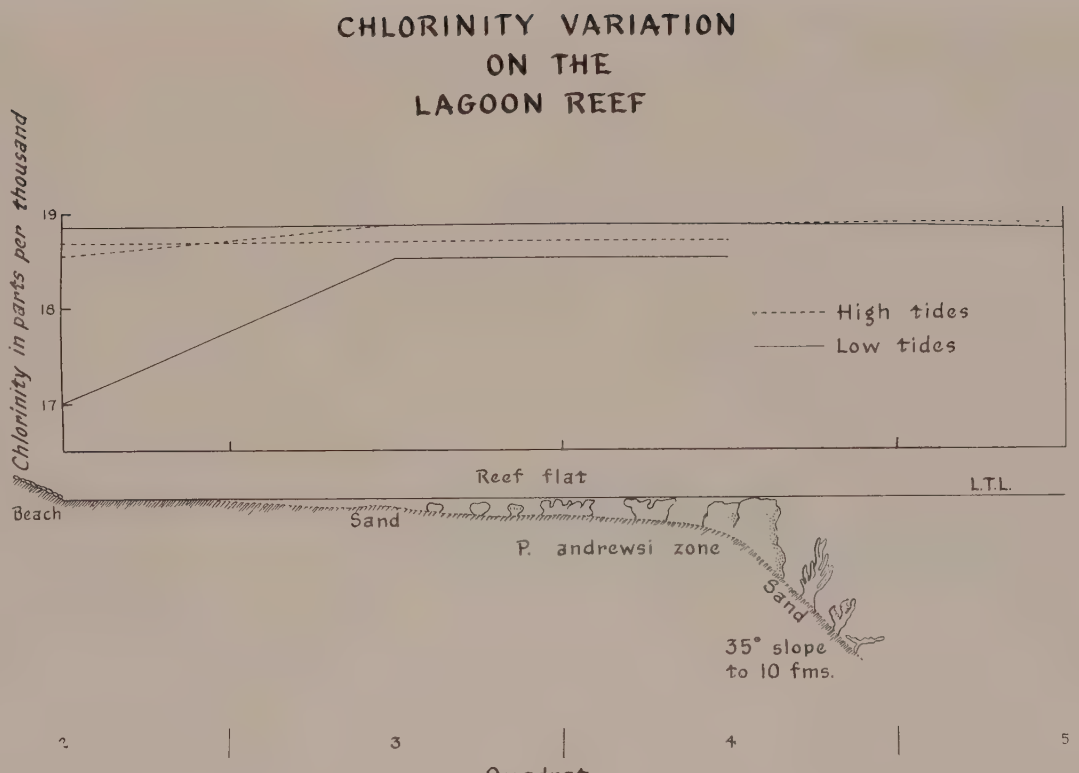
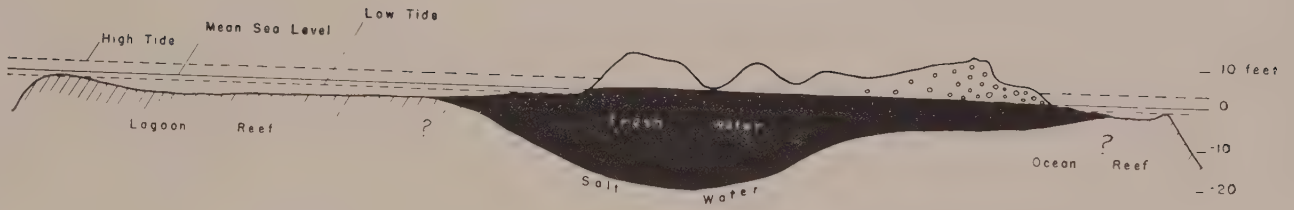

CHLORINITY VARIATION
ON THE
LAGOON REEF
Chlorinity in parts per thousand
19
18
17
High tides
Low tides
Reef flat
L.T.L.
Beach
Sand
P. andrewsi zone
Sand
35° slope
to 10 fms.
2
3
4
5
Quadrat

FIGURE 11



CROSS SECTION OF INE ISLAND SHOWING TOTAL DEPTH OF GHYBEN-HERZBERG LENS. VERTICAL EXAGGERATION 10X.

FIGURE 12

THE NATURE AND EVOLUTION OF THE HAWAIIAN INSHORE FISH FAUNA[1]

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Honolulu 14, T. H., U. S. A.

Introduction

A very large number of the shallow-water animals of the Hawaiian Islands are endemic, and the area is usually considered a major zoogeographic subdivision of the great, marine, shallow-water, Indo-West-Pacific region (Ekman, 1953). Among Hawaiian species of inshore fishes estimates of endemism range between approximately 15% (Fowler, 1928) and 50% (Jordan and Evermann, 1905). In my opinion the latter figure is about correct. Neither the distinctiveness of the Hawaiian inshore fish fauna nor its derivation from an Indo-West-Pacific stock are in doubt, and these matters will not be discussed here. The purpose of this paper is to present a preliminary analysis of the factors that appear to have brought about the present status of the Hawaiian shore-fish fauna. It is based on five years of investigation and on collecting trips to Johnston, Wake, and most of the Hawaiian Islands. Nevertheless, present knowledge is insufficient to provide more than leads to the subject, and no help is to be obtained from other components of the Hawaiian shore fauna, for they are more poorly known than the fishes. Consequently the ideas presented here can deserve no rating higher than that of working hypotheses.

Geography

The Hawaiian Islands (Fig. 1) form a long, narrow chain extending for some 1600 miles along a southeast to northwest axis. Hawaii, at the southern end of the chain is the largest island in the central Pacific, whereas Kure, at the opposite end, is the northernmost coral atoll in the Pacific. The greatest distance between any two islands in the chain is less than 200 miles. For zoogeographic purposes, Johnston Island (Fig. 1), about 450 miles to the south of the central part of the chain, must be considered an outlying component of the Hawaiian faunal area.

The nearest shallow waters to the north and east are those of the Aleutians and Alaska, somewhat less than 2000 miles away. Between

[1] Contribution No. 49, Hawaii Marine Laboratory in cooperation with the Department of Zoology and Entomology, University of Hawaii.

Hawaii and the mainland United States lie about 2000 miles of deep water. There are no shallow-water fishes common to the Hawaiian Islands and the shores of North America, though a few high seas species, e.g., the albacore, inhabit both regions. To the south of the United States, America slopes away from Hawaii, so that the distance from Hawaii to Panama is about 4000 miles. Thus the nearest American coasts with water temperatures similar to those of Hawaii are separated from that island by at least 2500 miles of deep sea. The Hawaiian fish fauna has derived little or nothing from tropical American waters.

The nearest shallow-water areas to the Hawaiian chain lie to the south and west. The Line Islands (Fig. 1) south of Honolulu extend to within about 850 miles of Hawaii and to 800 miles southeast of Johnston. Due west of Hawaii, Wake (Fig. 1), a northern outlier of the Marshalls, lies about 1200 miles southwest of Midway (and about half way between Honolulu and Japan).

Water Temperatures

With regard to surface-water temperatures, it can be seen from Table I that all of the islands of the Hawaiian chain, Wake, Johnston, and Palmyra (in the northern Line Islands) have about the same temperatures in summer; there is, however, considerable difference between the northern parts of the Hawaiian chain and the other islands mentioned in winter. Since the Hawaiian fish fauna is essentially a tropical one, it would seem probable that if any temperatures are critical for Hawaiian fishes, they are those of winter. Consequently summer temperatures will be dismissed from further consideration. In winter, the differences in water temperatures within the Hawaiian chain are greater than those between Hawaii and the northern Line Islands or Wake.

TABLE I

Surface Water Temperature (from Sverdrup, Johnson, and Fleming, Charts II and III)

	February		August	
	Temp. C.	Difference	Temp. C.	Difference
Wake	25	7	28	2
Midway	18	6	26	0
Hawaii	24	1	26	1
Johnston	25	1	27	0
Palmyra	26		27	

If cold water were the principal limiting factor in the differentiation of Hawaiian shore fishes, then greater variation would be expected within the Hawaiian chain than between the northern Line Islands or Wake and Hawaii. This, however, does not occur. On the contrary, the fish fauna throughout the Hawaiian chain seems to be quite homogeneous. Except for slight indications of racial differentiation in two species (*Kuhlia sandvicensis* and *Istiblennius zebra*), attempts to distinguish even races of fishes within the Hawaiian chain have been unsuccessful.

As to number of species, there are several *a priori* reasons for suspecting that it might differ at the two ends of the Hawaiian chain. One is the great differentiation in winter water temperatures within the chain noted above. A second is that the southeastern islands are high and volcanic, whereas the northwestern are low coral atolls. Presumably all the atoll habitats are present around the high islands; however, the volcanic rock habitats of the eastern islands are absent from the whole western part of the chain. Finally, there is the possibility that the low western islands have a larger fauna because they are older. Unfortunately, it is impossible to say whether the number of species at the two ends of the Hawaiian chain actually does differ, for the low leeward islands have been very poorly collected and the absence of species records from these islands means nothing. Consequently, it becomes necessary to fall back on general impressions for what they are worth. After collecting at most of the islands from Hawaii to Midway it is my personal feeling that there is a decrease, though probably not an evenly graded one, in the number of species from southeast to northwest. Indeed I have collected no species in the leeward islands that I had not already collected in the high Hawaiian islands. (On the other hand the number of individuals of a species seems to increase from south to north. Also, judging from a few non-commercial species, the maximum size of individuals within at least some species increases from south to north.) If, however, there are species on the high islands restricted to lava rock habitats, I do not know them. In Oahu, the great majority, at least, of the species found over lava rock may also be encountered over coral or coralline limestone areas. It seems necessary then to fall back on winter temperatures as the cause of the decrease in number of species in the leeward group, if indeed such a decrease really takes place.

Current Systems

The adults of most Hawaiian inshore fishes are bottom feeders that would presumably starve in the open sea. It is generally believed,

though by no means verified, that most such fishes arrived in Hawaii as planktonic larvae carried in by the ocean currents. Under such postulates the current systems should play a dominant role in the development of the Hawaiian inshore fish fauna.

However, there seems to be little correlation between the present current systems of the Hawaiian region and the fish immigration routes. Although the Hawaiian inshore fish fauna has come in from the south and west, there are no known northeasterly-flowing currents reaching the Hawaiian Islands (see Sverdrup, Johnson, and Fleming, 1942, Chart VII). The North Equatorial Current, in which Hawaii lies, flows toward the west, and the eastwardly flowing Equatorial Counter Current does not pass within 800 miles of these islands. If the derivation of the Hawaiian inshore fishes is to be tied in with present current systems there would seem to be only two possibilities, both in my opinion rather remote. One is that the larval forms were carried to the east of Hawaii in the Counter Current and then doubled back in a great eddy of the North Equatorial Current that carried them over 800 miles north. The other is that Hawaiian fishes were carried in to Kure and Midway from the Bonins and Japan by a southern tongue of the Kuroshio Current (see Sverdrup, Johnson, and Fleming, 1942: 122). This would involve transport in a slowly moving current across more than 2000 miles of deep water.

There is of course the possibility that the current systems of the Hawaiian area were different in the Pleistocene than they are today. However, Arrhenius (1952) has shown that there was little or no displacement of, at least, the Equatorial Counter Current during the Pleistocene.

If the immigration of Hawaiian fishes took place randomly, e.g., via occasional eddies within the major current systems, then it would seem logical that they came in from the nearest islands, namely, Johnston and Wake. There is indeed considerable evidence in the Johnston fish fauna that this island has formed something of a way point in both the immigration and emigration of Hawaiian fishes.

Nature of the Hawaiian Fish Fauna

Before treating the endemism among Hawaiian fishes it seems advisable to divide these into several ecological categories. First, there are a small number of Hawaiian fishes, mostly gobies, that have taken up a primarily fresh-water existence in the streams of the high islands. At the other end of the scale are the deep-water fishes, both bathypelagic and bottom dwelling forms. Third, there is a group in which the adults are pelagic, e.g., most sharks, the tunas, flying-fishes, and

Schindleria. All three of the above-mentioned groups will be omitted from further consideration in this paper. The remaining, inshore fishes will again be divided into two categories for purposes of treatment here. One group contains those species ordinarily living in depths of approximately 200 to 600 feet, and the other those fishes usually inhabiting depths of less than about 200 feet.

Of those fishes living between 200 and 600 feet, relatively little is known. In Hawaii there is a considerable hand-line fishery for such species, but only the larger forms are taken, and of these only the edible forms are brought in. About all that can be said of this group is that it seems to show less endemism and a greater affinity to the Japanese fish fauna than is present among shallower-water forms.

The rest of this paper will be devoted to those fishes usually living in less than 200 feet of water. This inshore group is not only the best known, but contains well over half of all the fishes recorded from the Hawaiian Islands. Four main features must be noted about these forms. First, a large proportion of the Hawaiian species appear to be endemic. Second, at most a very few of the Hawaiian genera are endemic. Third, the Hawaiian inshore fish fauna, in distinct contrast to the Hawaiian terrestrial biota, is a harmonic (balanced) one. Finally, the Hawaiian fish fauna does not appear to be a particularly impoverished one by Central Pacific standards.

It is necessary to discuss each of the above points in some detail. Since the lack of endemic genera and higher categories is most easily dismissed, it will be dealt with first. There are, to my knowledge, only two genera of Hawaiian shallow-water fishes (*Gregoryina* and *Microbrotula*) that can be considered endemic. However, both of these are made up of small, rarely collected forms that may have been overlooked elsewhere.

The statement that a large proportion of Hawaiian inshore species of fishes are endemic needs some qualification, for the question may be raised whether the Hawaiian endemics are not merely subspecies. Indeed it cannot be proved that these endemics will not and do not interbreed with their central Pacific counterparts (see below). Nevertheless, there are two basic reasons for considering the Hawaiian endemics as full species. One is that the Hawaiian representatives of an Indo-Pacific Artenkreis are usually better differentiated than any other populations of the Artenkreis, e.g., *Acanthurus sandvicensis* in the *A. triostegus* complex (Schultz and Woods, 1948). The other and more important reason is that no intergradation can be demonstrated at the borders of the ranges between the Hawaiian and the central Pacific forms. Johnston and Wake (Fig. 1) are both islands which geograph-

ically lie somewhat between the Hawaiian and central Pacific faunal areas, and a mixture of these two faunas (but with a high predominance of Hawaiian forms) is found at Johnston. Nevertheless, no intergradation between the Hawaiian endemics and their central Pacific counterparts was found at Johnston. Among four species complexes investigated in some detail (*Muraenichthys cookei- laticaudata, Gymnothorax eurostus-buroensis, Acanthurus sandvicensis-triostegus* and *Kuhlia sandvicensis),* the Johnston populations of the first three represented the pure Hawaiian stock, and of the fourth the pure central Pacific form. In no instance were *both* the Hawaiian and the central Pacific forms found at Johnston, nor have they been found together elsewhere.

As to the harmonic nature of the Hawaiian fish fauna, a very high proportion of the families and genera of shore fishes found in the central Pacific east of Samoa have representatives in Hawaii. There are, however, two notable and curious gaps in the Hawaiian shallow-water fish fauna. Throughout most of the tropical Pacific, two of the most prominent genera are *Lutjanus* and *Epinephelus.* In the northernmost of the Line Islands, for example, perhaps a half dozen species of each of these genera occur. In the Hawaiian Islands and Johnston *Lutjanus* appears to be totally unrepresented, and *Epinephelus* occurs only in a single, deep-water form. Yet the families to which these two genera belong are abundantly represented in Hawaii by other, smaller, deeper-water genera. These gaps seem especially peculiar in that both genera are made up of generalized, moderate to large sized fishes. Furthermore, both are present in Japanese waters that would seem to be colder than those of Hawaii.

With regard to the relative size of the Hawaiian fish fauna, little can definitely be said. On the one hand numerous central Pacific species are unrepresented in Hawaii. On the other, more inshore species are recorded from Hawaii than from any one central Pacific island group east of Samoa. However, except for Hawaii, no central Pacific island group east of Samoa has been adequately collected. Under any circumstances the Hawaiian inshore fish fauna, with some 400–500 known species, cannot be called depauperate.

Characteristics of Hawaiian Endemic Fishes

The characteristics by which Hawaiian endemic fishes differ from their ancestral forms, i.e., their central Pacific representatives, can best be treated under two categories: physiological and morphological. In morphological features the Hawaiian endemic fishes show no pattern of divergence from their central Pacific relatives. One Hawaiian endemic has a greater number of fin rays; a second has fewer gill rakers; a third has the dorsal fin originating farther forward; a fourth has

slightly longer jaws; a number differ in various details of color pattern; etc. Even within a family no trend of divergence is shown by the various Hawaiian endemic members. Consequently there seems to be no possibility of correlating the morphological peculiarities of Hawaiian endemics with distinctive features of the Hawaiian environment, e.g., colder water temperatures. One anticipated correlation of this sort is strikingly absent: Hawaiian coral reefs are notably dull colored as compared with those of the central Pacific, yet the fishes seem to be as brightly pigmented as those to the south. It is not, therefore, easy to escape the inference that the morphological peculiarities of Hawaiian endemic fishes are *per se* non-adaptive.

Treatment of physiological differentiation is handicapped by the fact that almost nothing is known of the physiological reactions of tropical marine fishes. It might be expected that Hawaiian fishes have become physiologically adjusted to life in colder waters than their more tropical ancestors. There is some indirect evidence for, and none against, this supposition. The best available bit of information in this connection is derived from the spawning seasons of certain Hawaiian fishes. One would expect (Ekman, 1953: 113) that a tropical species living in Hawaii would spawn, at least primarily, during the summer months when water temperatures most closely approximate those of its ancestral home. However, many Hawaiian fishes spawn primarily or entirely in winter at temperatures below those ever reached in more tropical regions. One example will suffice. Around Honolulu *Pomacentrus jenkinsi* spawns from December to March; no ripe females have been taken during the remaining months. At Arno, 10° nearer the equator in the Marshalls, Dr. Strasburg informs me that ripe females of this same species were taken in July. (Hawaiian specimens of *Pomacentrus jenkinsi* do not seem to be morphologically distinguishable from their central Pacific counterparts, which further emphasizes the advisability of treating the morphological and physiological peculiarities of Hawaiian forms separately.)

The restriction of spawning in *Pomacentrus jenkinsi* (and other similar examples can be given) to winter around Oahu is the reverse of what would be expected, and the explanation for this phenomenon is obscure. Perhaps the Hawaiian populations of *P. jenkinsi* have at one time become adapted to waters even colder than exist around Oahu today, e.g., in the Pleistocene, and the winter spawning is merely a holdover from such adaptation. (In any event, the Hawaiian fishes must either have come in since the Pleistocene, under which supposition it is difficult to explain the high degree of endemism, or they must have been able to survive the Pleistocene water temperatures of Hawaii.)

Causes of Endemism among Hawaiian Fishes

In the preceding sections the nature of the Hawaiian fish fauna has been discussed. It remains, however, to attempt some explanation to the question of why it is as it is. Why, on the one hand, has not the fish fauna undergone the adaptive radiation found in the Hawaiian terrestrial fauna (see Zimmerman, 1948)? Why, on the other hand, is there any endemism among Hawaiian fishes at all?

The answer to these questions would seem to be found in the degree of isolation of the Hawaiian Islands. Though the Hawaiian chain is equally isolated in space for both fishes and terrestrial organisms, the fishes (and other marine organisms) seem far better adapted to getting there than most terrestrial forms. This is immediately indicated by the balanced nature of the fish fauna as compared with the terrestrial biota. Indeed there are a large number of central Pacific fishes unrepresented in Hawaii, but on the whole it looks more as though such absences were due to unsuitable conditions for survival rather than to inability to arrive there.

If the foregoing is correct, most or all suitable inshore environmental niches have been filled by immigration, and the possibility of an adaptive radiation among Hawaiian fishes is precluded. But also, if the foregoing is correct, the question of why there is any endemism at all among Hawaiian fishes becomes difficult to answer.

In my opinion, the endemism among Hawaiian fishes has been brought about by two factors acting together and, in general, additively. The primary cause is a moderate degree of isolation. The secondary cause is the slightly colder water of the Hawaiian Islands as compared with the tropical central Pacific.

With regard to isolation, it has been stated above that the vast majority of central Pacific fishes have probably arrived in Hawaii at one time or another. By this it was not meant that the Hawaiian fish population is deluged by an influx of immigrants every year, but rather that one or a small number of specimens of most central Pacific species have managed to get to the Hawaiian chain from time to time. Presumably the original entrants, if they survived and reproduced, would have had time to saturate the islands with their descendants before the next immigrants arrived. There is some evidence that such a process does take place. As already noted, at Johnston the pure central Pacific stock of *Kuhlia sandvicensis* is represented, but among three other fishes the pure Hawaiian form is present. Apparently what has occurred is that in the case of *Kuhlia* the central Pacific stock has been able to saturate the island with its representatives. Subsequent immigrants from Hawaii were either unable to survive at Johnston, or if they

survived and interbred with the central Pacific form they did so in such relatively small numbers as to leave no trace of intergradation on the Johnston population. For the other three fishes the situation would seem to be similar except that the Hawaiian form has either arrived first or totally displaced the central Pacific type.

The original immigrants to Hawaii that gave rise to the descendant population may or may not have been aberrant members of the ancestral stock. Furthermore, during at least the initial increase in the population, random drift may have been effective. Finally, among Hawaiian fishes, these possibilities of aberrant initial immigrants and random drift may have occurred at least twice, once at the stepping stone of Johnston (or possibly Wake) and again on first arriving in Hawaii.

The above factors (more fully treated in Zimmerman, 1948: 122-125) may have caused differentiation through isolation *per se*, and this differentiation would be of a non-adaptive type. Once arrived in the Hawaiian Islands the fishes may have undergone further differentiation in adapting themselves to the colder waters of this area. (Evidence for one such adaptation has already been discussed.) Such adaptive physiological differentiation would increase the ability of the residents to compete with further immigrants, so that these would be able to pass even fewer genes into the resident population than before (providing of course that they could interbreed at all). Consequently, I believe that the integrity of a Hawaiian endemic species has been maintained whether or not an occasional central Pacific immigrant has interbred with it, and that the physiological adaptations of the endemics have insured them from extermination through competition with the central Pacific immigrants that may have arrived subsequently.

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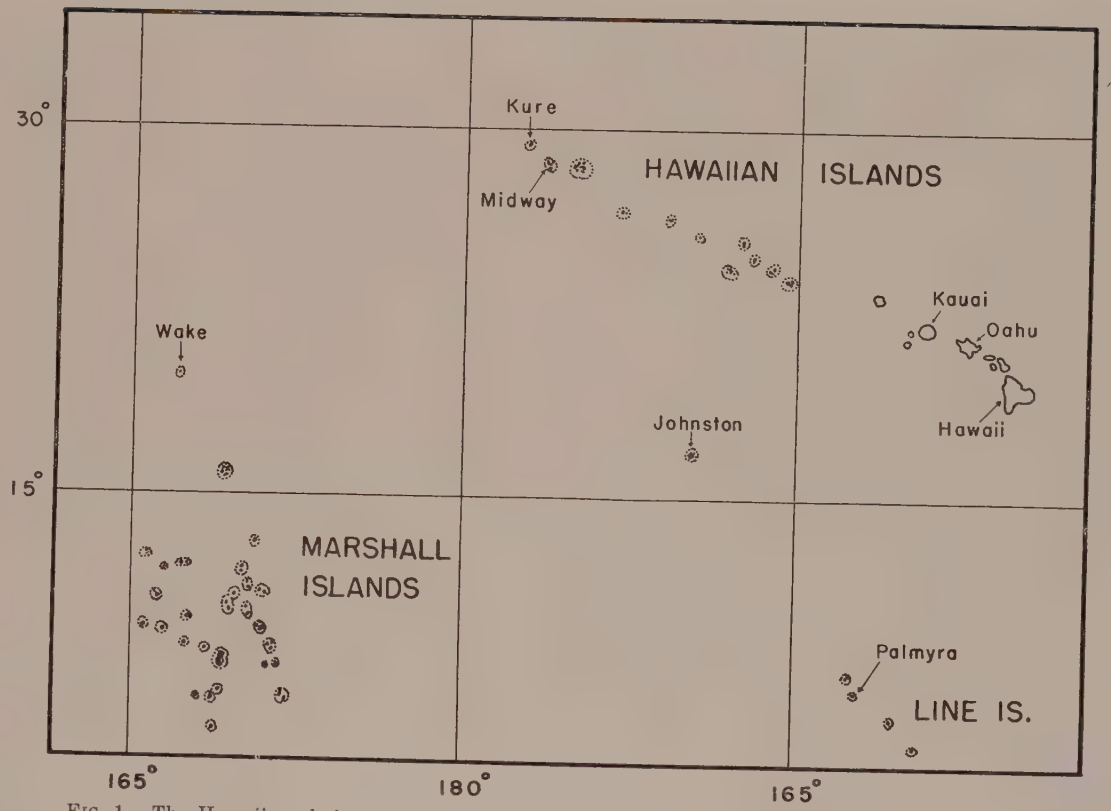

FIG. 1.—The Hawaiian chain and the islands nearest to it. Land areas greatly exaggerated in size.

AN ENUMERATION OF THE HYPOTHETICAL ROLES OF ALGAE IN CORAL ATOLLS *

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Our objective here is to enumerate the prospective roles of algae in atoll structure and biology. Essentially this is an enumeration of hypotheses developed from the observations of the Atoll Research Teams insofar as psychological observations were made. It is hoped that this enumeration will lead to testing by future work and that tenable theories of the roles of algae will thereby come to replace these hypotheses.

Flying over an atoll one sees a more or less complete ring of rock just barely submerged beneath the sea's surface. Closer inspection reveals in the water an increase in the size of the fish standing crop, zooplankton population and oxygen production as the atoll is approached and a position inside the lagoon attained. The sea edge of the atoll is solid stone. This edge, from the Carolines and Northern Marshalls to the Tuamotus, is essentially a pink mass, as is much of the water covered, flat, upper surface. The lagoon edge of the atoll ring may be a muddy shore with mangroves, a sandy shore, or a coelenterate coral dominated reef. On the atoll ring there are more or less extensive islands which protrude above high tide line. There is a distinct black area clearly visible in most aerial photographs wherever the above-tide shore is of solid material. The outermost edge of this black area can be taken as high tide line in interpreting aerial photographs. Where not covered with vegetation the surface of these islands, whether sandy, gravel or solid rock, is grey.

Each of the observations related above is apparently rather directly dependent upon algal populations. The hypothetical roles of these algae are of two major kinds, *physiological* and *mechanical*. The *physiological* roles are affected by the action or the utilization of metabolic products. The *mechanical* roles are structural, due to the physical nature of the products accumulating through the physiological activities of the algae. It is our intention here to call attention to these hypothe-

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tical roles as they are related to the observations on the general appearance of an atoll as described above.

Taking Darwin's hypothesis as a description of the way an atoll forms, we are able to open the first scene of the hypothetical roles played by the algae. The first stage setting is that of an igneous mountain, an island surrounded by an essentially homogeneous sea. The shores of this mountain could be expected soon to become coated with attached algal material. In this algal material there would develop and feed the host of animals to be found in the intertidal area.

The essential limiting salts for algal growth would become concentrated from the passing sea currents and from the rain water flowing down the mountain. Through this accumulation of materials, passed on to the animals that eat the algae, the concentration of the materials required for life would continually increase. This would result in an increasingly dense population. After a time this population is far more dense than that found either in the open ocean or on the newly cooled igneous mountain.

Perhaps largely due to the feeding of fish on fleshy algae below the low level of the tides, the algae that developed there are predominantly calcareous. These algae carry on photosynthesis, making an excess of food material and depositing calcium salts. The story of the reef that develops through the accumulation of calcareous material by the coral organisms, algal and animal, has been told often. But an aspect that has not been emphasized is the continuously cumulative nature of the process. Dead fragments are for the most part passed over the reef edge into the islands or into the lagoon. Continued sea reef edge growth is probably largely by accumulation of new material from the sea. Thus though the role of typhoons is argued, it seems very likely that otherwise atolls continuously increase in mass by the addition of newly accumulated material at their sea margins by the algae.

The primary role of the algae in the Central Pacific must be this removal of inorganic materials from passing oceanic currents. With the energy accumulated by photosynthesis inorganic materials are combined and passed on to other organisms as organic foods. As an example consider *Porolithon onkodes,* the reef covering calcareous alga.[1] For a crustose coralline alga it is peculiar in its ability to thrive in brilliant light and withstand brief exposure to the air. This species is eaten by parrot fish. The parrot fish defecate not only calcareous material but nutrient salts and thus enrich the water beyond the reef. Planktonic algae reincorporate this material into algal cells. Plankton feeders

[1] With variations in reef exposure and local topography other species of *Porolithon* may become locally abundant. They rarely become dominant. Rarely a *Goniolithon* appears in numbers but in the situations seen in the Tuamotus only on the reef flat well inshore.

can consume the algae, forage fish and then fish such as the wahoo (*Acanthocybium* sp.) and yellowfin tuna (*Thunus ablunga*) and finally the sharks may receive this material. This food pyramid extends away from the atoll. Thus may be outlined the hypothetical explanation of the increase in populations as an atoll or island is approached.

This great increase of fish provides food for the island peoples and, potentially, an income source for them. For example production of whole or ground dried fish might very well be developed as a new source of income to atoll peoples. Production of such a product would be feasible on a family basis and require little material beyond what is already available in any copra producing area.

The legal aspects of oceanic waters have an interesting relationship here. If one is concerned with the fish population near an atoll it would appear that its size would be due to land bound causes. The land owner could cite this in reference to a claim of ownership of the near-shore fish. It is to be noted in this connection that the Polynesian peoples did claim the waters that extended away from the shore of their particular piece of land.

The plankton from an open sea situation would not support the fauna found where it is the animals that dominate the reef: indeed on upwind atoll areas, where the surface currents strike predominantly, coral animals are usually at their fewest. Thus one is inclined to think that the benthic algae must have been the primary organisms of abundance of any new mountain appearing in the ocean. Indeed in Hawaii it is just these sessile algae that are the first organisms to become apparent on new lava flows. One is inclined to suspect it is through their concentration of nutrient salts from the passing sea and the passing of this concentration to locally developing photosynthetic unicells that accounts for coelenterate coral growth and distribution on an atoll-wide basis.

As noted above blue-green algae (or perhaps we should better say blue-green bacteria) grow on and in all surfaces near high tide line. The precise species concerned are considered in the "Reports of the Raroia Atoll Team" in the *Atoll Research Bulletin*. In North Carolina, U.S.A., the blue-green algae so solidly bind the sand that airplanes use these sandy places as practice landing strips. On a coral reef and particularly above high tide line undoubtedly such binding stabilizes the sand bars and binds new sand grains that chance to come to rest. It is through these activities that such a sand bar can be expected to become an island. Particularly in this case the algae have a major role in the establishment of the island as a landmass sufficiently permanent that the climax atoll biota may become established.

They are critical agents in the chain of events leading to the removal of other factors limiting to the terrestrial biota. The accumulation of these from the passing sea current has been discussed above. However, for the sake of argument, with the appearance of algally induced islands two more possibilities arise.

With an increase in size an island on an atoll may come to hold in its interior a body of fresh water. Sea water penetrates the island. This lens of fresh water floats up and down with the tides on top of the sea water and the fresh water in such a situation gradually become mixed with the salt water. Both the freshness and lateral extent of the fresh water lens is regulated by such factors as rainfall, island size, tide range and the permeability of the islands especially near their shores. It has been postulated that the algae play a part in the transformation of the sand at the shore into the beach rock and other atoll rock types.

Birds may use the islands as roosts. This effectively initiates a collection on the islands of nutrient salts from the fishing activities of the birds. Hatheway (*Atoll Research Bulletin*) and Fosberg (see in this volume) have discussed this in some detail in reference to the native vegetation. This seems to be a particularly important factor in the case of phosphate. It is to be noted that the fish phosphate got into the fish as phosphate hypothetically accumulated near the atoll by the sessile algae.

The story of available nitrogen is somewhat different. In part it duplicates the phosphate story. Perhaps in a native climax vegetation with a well developed humus beneath, the birds or bacteria might provide a replacement of available nitrogen lost to the sea. Where there is little or no humus, as in the hurricane-swept Tuamotus, the upper ten centimeters of sand and gravel has a dense layer of blue-green algae that may very well substitute for humus. In the coconut plantations and *Guettarda* groves the soil is largely gravel or nonhumified sand, with sand filling a few decimeters below the surface. Yet there is a root zone well developed.

There is no growth of legumes on these atolls that could account for nitrogen fixation by species of *Rhizobium*. The soil is exceptionally aerobic and of carbonate buffered pH's. There are but few bacteria known (e.g. *Azotobacter* and the *Itersonia* described recently by Derx) that fix nitrogen under these conditions. Bortels, Gerloff, as well as Fogg and others have investigated the nitrogen fixing properties of blue-green algae. It is found that many related to those dominating the

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[2] *Lithothamnion* is a very unusual alga on the reefs in the Tuamotus and it is not likely to be common on any reef edge in the habitats where ordinarily *Porolithon* is found. The two generic taxa are very distinct, belonging to separate sub-families of the Corallinaceae. Certainly "Lithothamnion ridge" should not be used for the ridge of algal materials which often appears near the seaward edge of sea reef flats.

atoll soils do fix significant amounts of nitrogen. This problem is under investigation at present and preliminary cultural experiments lead us to believe that apparently the soil formed from atolls are significant nitrogen fixers.

The algae cover most of the surface of the atoll and wherever this surface is marine, marine organisms live on the algae. The case of parrot fish feeding on coralline reef formers has already been noted above. Sea urchins at the surface and the countless numbers of boring echinoderms, worms, and other organisms are by their homemaking and feeding habits induced to destroy the algae of the reef and reef flat.

Much attention has been paid to the black zone at high tide line where algae in cooperation with exfoliation must be the principal causes of destruction of the rocks at the seaward edges of the islands. Gastropods of the genera *Littorina, Meloraphe, Tectarius* and their herbivorous cohorts feed on the blue-green algae of the black zone. By their rasping of the algae they remove rock material. The surfaces above high tide on which the algae are growing are softer than those freshly exposed or where no algae are growing. To a certain extent the algae grow into the carbonate rocks. Their pigments diffuse into the rocks and become absorbed. It is most likely that it is the chemical results of these processes that are the causes of the observed softening.

It has been postulated that the softening noticed beneath the black algal coating is an evidence of solution and again of deposition. It seems more likely that it is an evidence of solution aided by the pH variations, especially at night, and the considerable amounts of heat accumulated and thus higher mobilities (but not of carbonate) under the black layer of algal material.

The only reefs seen by the present author to be predominantly coelenterate reefs are those in the lagoons. Such developments are reduced toward the windward side of the atoll and in the more closed atolls. The algae very definitely play a part both in the development and in the death of these animals. Firstly they play a role as the food, directly as phytoplankton or zoozanthellae or indirectly as food for the zooplankton, which in turn may form the food of the coelenterate corals. What is said here is true also of the sea reef corals and those of greater depths.

The edges of lagoon reefs are coelenterate corals at Raroia, elsewhere in the Tuamotus and elsewhere in comparable situations, e.g. at Johnston Island and in Kaneohe Bay on the island of Oahu in Hawaii. Often species of *Pocilopora* or *Porites* form the uppermost surface near the edge as a series of flattened branches condensed behind the reef edge almost into a pavement.

The upward limit of growth for the coelenterate corals on these reef edges seems to be regulated by their tolerance to air exposure. Some algae seem to tolerate air exposure better than the coelenterate corals and grow on the uppermost, often inactive, coelenterate tips just a decimeter or less from the reef edge. This must interfere with the feeding of nearby polyps especially on the side of the algal patches away from the reef edge.

Between the closely arranged coelenterate branches, as the reef extends, circulation must be cut down. In such places algae such as *Rhipilia geppii* grow. They come to fill the spaces between the branches as do such algae as *Zonaria variegata*. This later may extend over the top of the coelenterates a few decimeters in from the edge of the reef so extensively as to mask the coelenterate corals completely. The coelenterate corals become weak structurally and break off; so that in from the edge of the reef there is a lower area, a pool area. In this lower area and on the higher parts of shoreward areas of shore-bound reefs, crustose coralline algae again come to predominate in some localities, especially where the gravel or reef fragments are large.

In any intertidal region, or above on a lagoon shore, the blue-green algae play the same roles as they do on the sea shores already described, but more often on gravel rather than on conglomerate material.

The lagoon bottoms of some atolls are essentially meadows of the green alga *Halimeda*. At Raroia, in the Tuamotus, no dredge haul indicated living *Halimeda* on the bottom. However, there the sides of the reef patches in the lagoon are often clothed with this genus and *Caulerpa*. The latter, as *Caulerpa bikinensis*, is a favorite food of the turtles.

Halimeda is apparently one of the major contributors to the sediments that in turn contribute to filling the lagoon. The calcium salts *Halimeda* deposits are accumulated from the lagoon waters: the other materials filling lagoons come chiefly from outside the lagoon or from alga-smothered dead, or fish consumed, coelenterate corals within the lagoon.

Lastly a word about algae and man. The algae form an important part of the diet of the Oriental, and formerly of the Polynesian peoples wherever available. They are not important apparently in any other way directly, nor are they important to other Pacific people at present nor apparently were they in the past directly. They are, however, prospectively of great importance to these people as a major primary cause of their food and land.

HAWAII MARINE LABORATORY

CONTRIBUTIONS

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25 humans are known to have been stung as a result of handling cone shells—and 5 have died! Conus geographus and Conus textile have caused all the fatal cases. None of the 5 Hawaiian cases has been fatal.

Recent Cases of Human Injury Due to Venomous Marine Snails of the Genus *Conus*

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THIS paper summarizes information on a number of recent cases of injury inflicted upon humans by marine snails of the circumtropical genus Conus.

Accounts of the five previously unreported instances of humans stung by Conus in the Hawaiian Islands are included. Four of them were due to three species of Conus not previously known to be toxic to man. Cases in other areas of the Indo-West Pacific region have been reported in scattered publications. Only one reported occurrence since the last general summary (Hermitte, 1946) has come to my attention. It is discussed below. Notes on previously unreported cases from Australia, Kwajalein (Marshall Islands), and Guam (Marianas Islands) are also included.

Biology and Venomology of Conus

The genus Conus is with a few exceptions confined to the tropics, where its representatives are most abundant in shallow water. Members of this genus are the most abundant large marine snails in many areas of the Indo-Pacific region. They are characteristically associated with coral reefs. Twenty or more species of Conus are often present on a single reef or other confined area.

The animals are typically nocturnal, burrowing in sand or under stones or coral during the day and actively crawling about at night, often apparently in search of food. All members of the genus are believed to be predatory carnivores. I have studied the feeding habits of about 30 species. Most species feed on annelid worms, some feed on other snails, and a few feed on small fishes (Kohn, 1956).

The sting of Conus is normally used by the mollusk to paralyze prey organisms prior to feeding. The operation is accomplished by injection of a detachable radula tooth, accompanied by venom. The process is described by Kline (1956) and Kohn (1956). The venom is probably a neurotoxin. Its chemical nature is unknown but is under investigation.

Several recent authors have discussed the venomousness of Conus, though not reporting new cases of human injury. Hiyama (1943) lists the species of Conus known to have stung humans according to the "presumed strength" of the venom. The most venomous species listed is C. tulipa Linné, followed by C. geographus Linné, C. textile Linné, C. aulicus Linné, and C. striatus Linné. However, C. geographus has been responsible for more reported human fatalities than any other species, while no fatality has been reported from the sting of C. tulipa. C. geographus is also the largest of those mentioned, attaining a length of six inches. It therefore probably delivers a larger quantity of venom with each injection than does C. tulipa, which reaches a length of about three inches. The single recent C. tulipa sting reported below caused only minor discomfort. It is the present writer's opinion that C. geographus is probably the species most dangerous to humans.

Conus textile and C. aulicus may each exceed four inches in length. The writer has not observed living C. aulicus, but individuals of C. textile are extremely active animals. Since stings of C. textile have been fatal in two reported cases, it should be placed high on the list of the more venomous species. Previous to Hiyama's (1943) list, C. striatus

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had not been reported to be toxic to humans. It is, however, rather related to the C. geographus group and has an extremely well developed venom apparatus and radula tooth (Clench and Kondo, 1943; Kohn, 1956).

DR. KOHN

Although shell size has been correlated with venomousness in the preceding discussion, such a generalization is not valid for the entire genus. For example, Conus leopardus (Röding), one of the largest species in the genus, may reach a length of nine inches. However, this species is characterized by a rather poorly developed venom apparatus with a radula tooth only about one-tenth as long as those of C. geographus, C. striatus, and C. textile. Observations on living specimens revealed that the prey is not stung prior to feeding but engulfed alive. Such a species, despite its size, is likely to be of little danger to man.

Cotton (1945) listed species of Conus previously known to have stung humans, and Hubert (1948) discussed the species which are likely to be dangerous in the Indonesian area. Fish and Cobb (1954) reviewed the geographical distribution of the previously reported occurrences.

Cases Prior to 1946

Hermitte (1946) summarized the 16 cases of Conus stings reported previously. These were inflicted by five species, as follows: C. aulicus, one case, not fatal, in the Moluccas; C. textile, five cases in Melanesia, two of which were fatal; C. marmoreus Linné, one case, not fatal, in the New Hebrides; C. tulipa, three cases in New Caledonia and the Tuamotu Islands, none of which were fatal; C. geographus, five cases in New Britain, Fiji, Japan, and Australia, of which three were fatal.

A previously unreported non-fatal case is described in detail by Hermitte (1946). The wound was inflicted by Conus geographus in the Seychelles Islands. It is the only known case from the Indian Ocean.

Recent Reports

Petrauskas (1955) has reported on a recent case from Manus Island, New Guinea.[1] On August 27, 1954, at about noon, a girl about eight years of age was stung by Conus omaria Hwass, a species not previously known to be toxic to humans. The victim collapsed after being stung in the thenar region. The wound appeared as a black spot surrounded by a moderately swollen area. Examination showed slurred speech, complete palsy, shallow breathing, absence of reflexes in the lower legs, arms and neck, partial affection of hand muscles, and complete freedom of face muscles. The heart rate was higher than normal. The temperature was not elevated. Complete paralysis of the respiratory muscles followed. Breathing was maintained by Silvester's method and later by oxygenation by means of an endotracheal tube and portable anaesthesia apparatus. Vitamin B_1 (100 mg) and penicillin (200,000u.) were administered. In two hours the patient had regained consciousness. Gradual recovery of respiration followed, but artificial respiration was continued for two more hours. The patient had completely recovered by the following morning. No after-effects were mentioned.

Conus omaria, a species related to C. textile, should thus be included as one of the species most dangerous to humans. The sting in the case cited would probably have been fatal in the absence of medical attention. Petrauskas (1955) also states that he was informed that other, unreported cases of fatal Conus stings have occurred on Manus Island.

Hitherto Unreported Cases from the Hawaiian Islands

An adult male was stung by specimens of Conus obscurus Sowerby (Fig. 1a) while collecting shells at Kahuluu, Hawaii, and Nanakuli, Oahu, on April 20, 1947, and April 29, 1956, respectively. These are the first known instances of human injury due to C. obscurus, a species which, although rather small, is closely related to C. tulipa and has a large and well developed venom apparatus. On both occasions a sharp stinging pain was experienced. Local edema, redness, and swelling followed and lasted for about one hour. The effect was described by the victim as similar to the sting of a centipede. Treatment consisted of vigorous rubbing and squeezing to express serum. No after-effects were noted.

In June, 1955, an adult female was stung in the thenar region by a specimen of Conus nanus Sowerby (=C. sponsalis Hwass?) (Fig. 1b) while collecting shells at Waianae, Oahu. This is the first known case of human injury due to this species, which is small and does not have a large venom apparatus. An intense stinging sensation, localized at the point of puncture, lasted about

[1] The writer is indebted to Dr. Alison Kay, who called his attention to this report and provided additional information.

FIG. 1.—Shells of species of Conus responsible for human injuries in the Hawaiian Islands. *a*, Conus obscurus Sowerby; *b*, Conus nanus Sowerby (= C. sponsalis Hwass); *c*, Conus textile Linné; *d*, Conus pulicarius Hwass. *a* and *b* 2X natural size; *c* and *d* natural size. Photographs by Mr. C. Sueishi.

one hour. No other effects were noted, and the wound was not treated. A red spot still marked the site of the puncture, however, 18 months later.

On July 5, 1956, an adult male was stung by a small specimen of Conus textile (Fig. 1c), about one inch in length, while collecting shells at Nanakuli, Oahu.[2] Site of the wound was the second joint of the left forefinger. The wound was lanced to enhance bleeding. Blood was sucked from the wound. The victim complained of immediate shortness of breath and developed a severe headache after 15 minutes. Extreme nausea followed and lasted 12 hours. Severe stomach cramps followed in the evening, some nine hours after the sting. The victim received no medical attention and was in normal health the following day, except for localized pain at the site of puncture.

[2] The author is grateful to Mrs. R. M. Gray, who supplied this information. A report of this case appeared in the *Hawaiian Shell News*, mimeographed publication of the Hawaiian Malacological Society, v. 4, no. 10, p. 103, Aug., 1956.

On November 13, 1955, at Nanakuli, Oahu, an adult male was stung on the finger as he reached into the sand to pick up a partially buried specimen of Conus pulicarius Hwass (Fig. 1d). A stinging sensation, which lasted for about one-half hour, and slight bleeding were reported by the victim. The injury was not treated and no other effects were noted.

Hitherto Unreported Cases from Kwajalein, Marshall Islands

No cases of human injury due to Conus have been previously reported from the Marshall Islands. In April, 1954, an adult male was stung by a specimen of C. tulipa while collecting shells at Kwajalein. The sting, in the right thenar region, was described by the victim as similar to a bee sting. The pain lasted five to ten minutes. The wound was not treated and no ill effects followed.

Hitherto Unreported Case from Guam, Marianas Islands

An incomplete report of an adult male stung by a specimen of Conus lividus Hwass on Guam in April, 1957, has been received in a letter from Mr. H. T. Ward. The injury was inflicted on the finger and was followed by severe pain throughout the entire arm for some time afterward. No additional details could be obtained. C. lividus was not previously known to be toxic to man. It is not closely related to any of the other species cited in this report.

Hitherto Unreported Case from Australia

While at Hope Island on the Great Barrier Reef in 1948, an adult female was stung on the thigh by a specimen of Conus which was being carried in a pocket.[3] The species responsible was not determined, since specimens of C. textile, C. marmoreus, and C. nussatella Linné were being carried in the same pocket. The site of the sting was marked by a raised inflamed area which persisted for two weeks. A severe headache followed the stinging but was apparently of short duration. If the area of the sting came in contact with salt water, extreme pain and throbbing resulted. No medical care was available. Recovery was complete after two weeks.

Symptomatology of Conus Stings

Symptoms associated with the cases reported and cited herein vary considerably. Hiyama (1943) describes the effect of the sting without specifying the species to which he refers. "The sting leaves a hole like that pierced by a needle, and a space around it as large as a copper penny turns purple. The venom spreads through the body rather rapidly, and cases are known in which death resulted within an hour after being stung." (Hiyama, 1943. Quoted from English translation, 1950.)

Halstead (in press) has recently summarized the symptoms which may be expected from Conus stings as follows: "Localized ischemia, cyanosis, and numbness in the area about the wound, or a sharp stinging or burning sensation are usually the initial symptoms. The presence and intensity of the pain varies considerably from one individual to the next. Some state that the pain is similar to a wasp sting, whereas others find it excruciating. Swelling of the affected part usually occurs. The numbness and paresthesias begin at the wound site and may spread rapidly, involving the entire body, particularly about the lips and mouth. In severe cases paralysis of the voluntary muscles is initiated early, first by motor incoordination followed by a complete generalized muscular paralysis. Knee jerks are generally absent. Aphonia and dysphagia may become very marked, and distressing to the victim. Some patients complain of a generalized pruritus. Blurring of vision and diplopia are commonly present. Nausea may be present, but gastro-intestinal and genito-urinary symptoms are usually absent. Coma may ensue, and death is said to be the result of cardiac failure. The exact cause of death is unknown.

Two of the more severe cases reported above differ from this description in that respiratory distress was marked. In the case reported by Petrauskas (1955), death due to respiratory rather than cardiac failure apparently would have ensued had the victim not received artificial respiration. None of the victims of Conus stings in Hawaii reported paralysis, dysphagia, visual or auditory symptoms, or generalized pruritus. Other symptoms mentioned in Halstead's summary were present in varying degrees.

Danger to Humans

The danger of human injury due to Conus is greatest in the Indo–West Pacific region, where the most venomous species are of widespread occurrence. Since the venom apparatus is a characteristic feature of this genus, all species are potentially dangerous to humans. However, certain species have particularly well developed venom apparatus and radula teeth. These species are often characterized by relatively thin shells with wide apertures, which may flare basally (Figs. 1a, 1c).

[3] An account of this case is given in a letter from the victim to the Hawaiian Malacological Society and included in the *Hawaiian Shell News*, v. 5, no. 1, p. 4, Nov., 1956.

The living animals should be handled, if necessary, with special care.

The most effective immediate treatment of Conus stings is probably lancing of the wound and removal of as much venom as possible by suction.

Summary and Conclusion

Eight hitherto unreported cases of human injury due to stings of marine mollusks of the genus Conus are discussed. Five of these occurred in the Hawaiian Islands, one in the Marshall Islands, and one in the Marianas Islands. No cases of Conus stings have previously been reported from these three archipelagoes.

Five of the cases reported were caused by four species of Conus not previously known to be toxic to man. All species of Conus are potentially dangerous to man, but certain species are particularly venomous. The shell characteristics of these species are noted.

A total of 25 cases of Conus stings are now known. Of these, five, due to C. geographus and C. textile, were fatal. The symptoms resulting from Conus stings vary considerably. A complex syndrome may be present. Immediate lancing of the wound and sucking of the venom from it are recommended. Further treatment is symptomatic.

Addendum

Since this manuscript was submitted for publication, a second case in the Seychelles Islands has come to the attention of the author. In February, 1957, a 10-year-old girl was stung on the fourth finger of the right hand by a specimen of Conus imperialis Linné. The incident occurred at Beau Vallon, Mahé Island. Severe pain at the site of the sting was experienced immediately but diminished during the next hour. The victim was taken to a hospital, where an incision was made in the wound to enhance bleeding. The patient was not detained at the hospital and was fully recovered the following day.

Summario in Interlingua

Octo nove casos de piccatura per conchas del genere *Conus* es addite al 17 que esseva previemente reportate. Cinque occurreva in Hawaii, un in le insulas Marshall, e un in le Marianas. Nulle caso de piccatura per *Conus* habeva previemente essite reportate ab iste archipelagos. Un nove caso occurreva in Australia.

Cinque del casos reportate esseva causate per quatro species de *Conus* non previemente recognoscite como toxic pro homines: *Conus lividus, C. pulicarius, C. obscurus,* e *C. nanus.* Nulle de iste casos esseva mortal.

C. geographus ha causate tres casos mortal, *C. textile* duo. *C. omaria* ha causate un caso quasi mortal. Le remanente casos esseva dolorose e vexante sed non seriose.

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Catch Rate, Size, Sex, and Food of Tunas and other Pelagic Fishes taken by Trolling off Oahu, Hawaii, 1951-55

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UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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CATCH RATE, SIZE, SEX, AND FOOD OF TUNAS AND OTHER PELAGIC FISHES TAKEN BY TROLLING OFF OAHU, HAWAII, 1951-55

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NOTE: This report is also Contribution No. 96 of the Hawaii Marine Laboratory, University of Hawai

ABSTRACT

Catch rate, lure preference, size, weight-length relation, sex and maturity, and food data are presented for the following species of fish: skipjack, little tunny, yellowfin, and dolphin. Where available similar data are reported for frigate mackerel, wahoo, and jack. These fishes were caught by trolling off the northeast coast of Oahu, Hawaii, during the period 1951-55.

The following inferences were made: The highest availability for all species combined occurred from May to October; no lure preference was found; the presence of one to several size groups was indicated by the weight distributions for these fishes; weight-length relations indicated a sexual difference in dolphin but not for the others; sex ratios showed a preponderance of males for skipjack, equal proportion of both sexes for little tunny and yellowfin, and a preponderance of females for dolphin, although considerable diversity of sex ratios between schools was observed for the latter; gonad examinations revealed mature specimens for all species except yellowfin; food of these troll-caught fishes consisted primarily of fishes, cephalopods, and crustaceans; interspecific competition for food was indicated.

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CATCH RATE, SIZE, SEX, AND FOOD OF TUNAS AND OTHER PELAGIC FISHES TAKEN BY TROLLING OFF OAHU, HAWAII, 1951-55

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During 1951 to 1955 inclusive, the 46-foot research vessel Salpa (frontispiece) of the Hawaii Marine Laboratory, University of Hawaii, conducted trolling operations off Kaneohe Bay on the windward or northeast side of Oahu. Fishing was undertaken primarily to stock the ponds of the Coconut Island laboratory with tunas and other pelagic fishes for studies of their response to stimuli. The studies were supported by three annual contracts (June 1951 to May 1954) between the University of Hawaii and the Pacific Oceanic Fishery Investigations.

Incidental to the fishing operations, data were accumulated on the catch rate, size, sex, and food of the several species which were caught. The data are summarized in this report primarily to make them available to other research workers studying the biology of these pelagic species. Such inferences as may be made concerning the life histories of the fish are presented briefly and should be considered tentative in view of the paucity of data in many instances.

The species discussed in this report are referred to by their common English names as follows:

Scientific name	English name
Katsuwonus pelamis	Skipjack
Euthynnus yaito	Little tunny
Neothunnus macropterus	Yellowfin
Auxis thazard	Frigate mackerel
Coryphaena hippurus	Dolphin
Caranx stellatus?	Jack
Acanthocybium solandri	Wahoo

METHODS

Fishing methods used in 1951 have been described by Tester (1952) and, in general, did not change in subsequent years. During 1951 and 1952, ordinarily six cotton trolling lines were used, two (about 90 feet in length) from each of two outrigger poles, and one (about 30 feet) from each side of the stern. During 1953 to 1955, the four outrigger lines were used, but for the most part only one line was trolled from the stern and, on infrequent occasions, even this was omitted. Since the catch rate is calculated as the average catch per hour rather than per line per hour[1/] this introduces a bias in year to year comparisons, but this is considered insufficient to account wholly for the changes in catch rate to be noted later.

Stainless-steel leader wires (about 6 feet in length) were snapped to the ends of the cotton lines. To these were attached a variety of lures. Trolling was conducted at a speed of about 6 knots, which kept the lures on or near the surface. After capture, viable fish were retained in the ship's livewell.

Fishing was confined mostly to the morning hours, with the ship leaving the dock at the Coconut Island laboratory between 0700 and 0800 hours and returning between 1200 and 1300 hours. On clearing Kaneohe Bay we would usually bear towards Mokumanu Island (fig. 1) and then head out to sea, the course being governed by such bird flocks as were sighted within the area of fishing. Often the ship would circle under a working bird flock for several minutes. Whenever strikes were made, trolling would be continued in the vicinity until there was reasonable assurance that no further catches could be made. Occasionally trolling would be terminated, even though more fish could have been caught in the vicinity, in order to avoid delay in transporting viable fish to the ponds at Coconut Island. The general fishing area, shown in figure 1, was

[1/] Accurate data on number of lines used per trip are not available in some years.

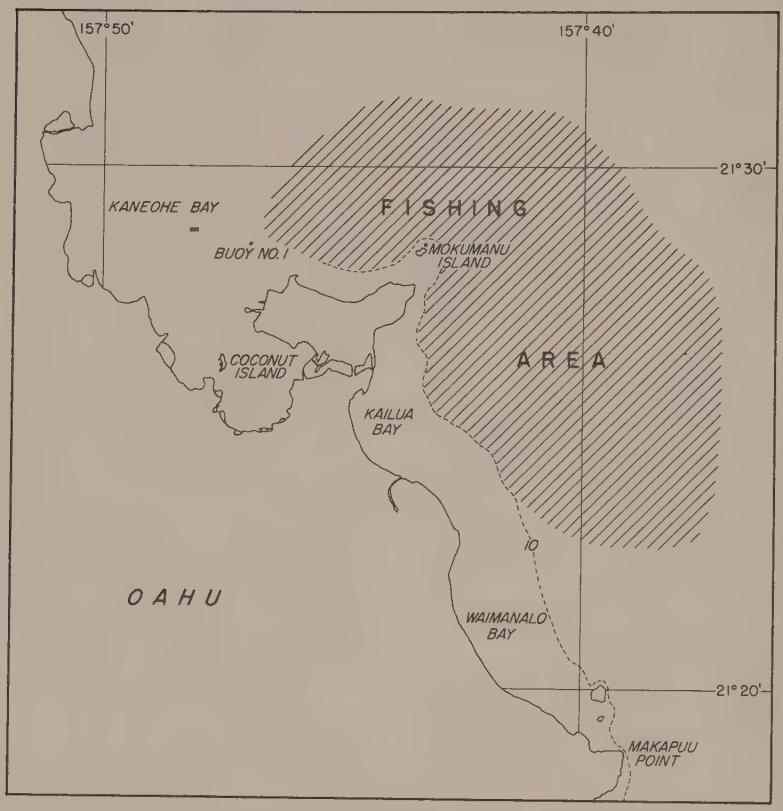

Figure 1.--Fishing area off the northeast coast of Oahu, Territory of Hawaii.

about 4 miles wide and 10 miles long. Only rarely was there fishing outside of this area.

In July and August 1953, 148 skipjack were caught by pole-and-line fishing, using nehu (Stolephorus purpureus) as live bait. These fish were not included in the analysis of troll catch rate, although they were used in certain other analyses in this report.

Fishing was done mostly during the summer months, but some effort was expended in other months of the year. The fishing periods for each year were as follows:

Year	Months of fishing
1951	January to October
1952	June to August; October
1953	June to August
1954	June to December
1955	January; May; July to September

At the laboratory the following determinations were made on the non-viable fish:

length--distance in centimeters from the tip of the closed jaws to the fork of the tail as determined on a measuring board (Hiatt and Hamre 1945)

weight--measured to the nearest one-fourth pound on a dial-type spring scale with weighing pan

sex and maturity--from examination of gonads, with gonad stage indicated by 1, immature; 2, maturing; 3, nearly mature; 4, ripe; and 5, spent

food--stomach contents were removed and preserved in jars containing 10-percent formalin; later, the total amount of food was determined volumetrically, the organisms were identified to the most convenient

exclusive category and their percentage by volume of the total was estimated. The number of each type of food item was also recorded. In cases where the fish had gorged itself on a large number of small organisms the number was recorded as "numerous."

For those species which were brought back alive and released into the pond, mostly little tunny and yellowfin, the only sampling data available is an estimate of the weight of each fish. As the estimates are considered reasonably accurate, they are included in the analysis of the weight composition of the catch.

CATCH AND CATCH RATE

Data on the catch according to year, month, and species are included in table 1. In the following discussion our results will be compared with those of Welsh (1949b), who conducted trolling operations in the waters between Hawaii and Kauai, including the present fishing area, from March 1948 to December 1948 and from February 1949 to June 1949. He used both the Salpa and the Territorial Division of Fish and Game's research vessel with fishing methods essentially similar to ours.

Table 1 shows that most of our catch (96 percent) was comprised of four species: skipjack, little tunny, dolphin, and yellowfin. Frigate mackerel, wahoo, and jack made up the remainder. The following tabulation of percentages shows that the same four species formed the bulk of the catch during the earlier period (Welsh 1949b), but that little tunny were of much greater relative abundance:

Species	1951-55	1948-49 (Welsh 1949b)
Skipjack	32.7	8.4
Little tunny	23.7	63.9
Dolphin	23.0	18.4
Yellowfin	16.8	4.2
Frigate mackerel	2.6	-
Wahoo	1.0	3.7
Jack	0.2	1.5

Annual variation in catch rate for all species combined and for each of the four principal species is shown in figure 2. The comparison between years is subject to error because of the variation in number of lines used and because of the variable seasonal coverage.

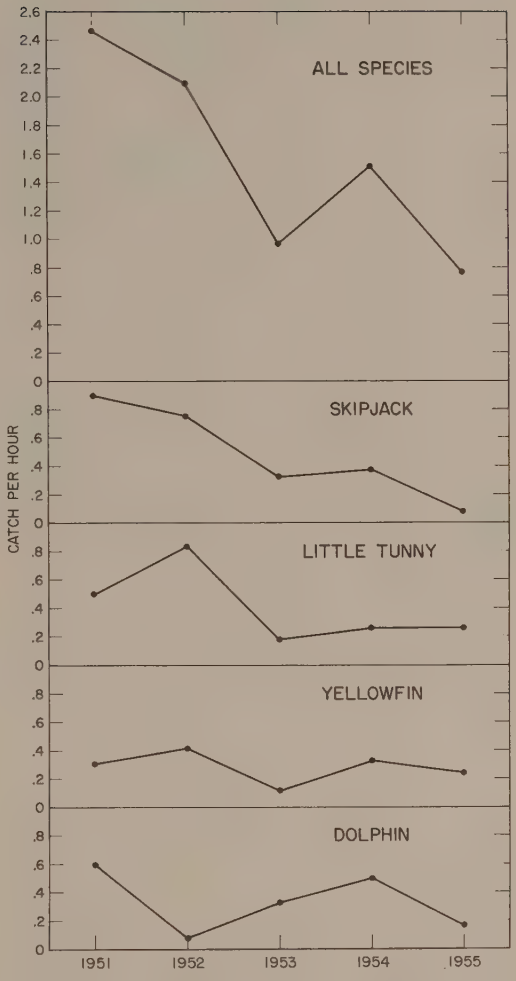

Figure 2.--Average annual catch per hour's trolling for all species combined and for the four most abundant species in the catch over the period 1951 to 1955.

The apparent availability of fish to trolling declined from a high of 2.46 fish per hour in 1951 to 0.97 in 1953, increased to 1.51 in 1954 and then decreased sharply to 0.77 in 1955. The apparent general decline following 1951 persists when the analysis is confined to comparable data for the summer months. The general trend can be attributed chiefly to a decreasing availability of skipjack over the entire period and a low availability of little tunny from 1953 to 1955. These apparent changes, however, may well be

Table 1.--Summary of catches, effort, and catch rate by species, month, and year

Year	Month	Number of trips	Number caught								Number of trolling hours	Mean catch per hour
			Skipjack	Little tunny	Yellowfin	Frigate mackerel	Dolphin	Wahoo	Jack	Total		
1951	Jan.	2	-	-	-	-	-	-	-	-	3.75	0.00
	Feb.	3	2	-	-	-	1	-	-	3	6.50	0.46
	Mar.	3	1	-	-	-	1	-	-	2	7.50	0.27
	Apr.	4	3	-	-	-	6	1	-	10	13.75	0.73
	May	8	23	5	-	1	24	-	1	54	21.50	2.51
	June	9	38	9	14	12	13	-	-	86	22.75	3.78
	July	8	24	3	4	1	16	-	-	48	20.25	2.37
	Aug.	9	38	15	3	4	23	-	-	83	24.75	3.35
	Sept.	8	19	21	14	4	2	1	-	61	21.75	2.80
	Oct.	6	2	30	16	-	13	-	1	62	23.75	2.61
Total		60	150	83	51	22	99	2	2	409	166.25	2.46
1952	June	10	21	8	6	-	5	-	-	40	23.50	1.70
	July	12	29	29	19	-	-	2	-	79	33.50	2.36
	Aug.	10	12	29	8	-	1	-	-	50	21.25	2.35
	Oct.	1	-	-	1	-	-	-	-	1	3.00	0.33
Total		33	62	66	34	-	6	2	-	170	81.25	2.09
1953	June	13	18	8	7	-	10	-	-	43	32.50	1.32
	July	9	4	4	3	-	16	-	-	27	29.25	0.92
	Aug.	5	5	3	-	-	1	-	-	9	19.75	0.46
Total		27	27	15	10	-	27	-	-	79	81.50	0.97
1954	June	7	5	13	-	-	7	-	-	25	17.50	1.43
	July	6	3	3	1	-	41	-	-	48	17.00	2.82
	Aug.	10	21	5	8	-	10	3	-	47	29.50	1.59
	Sept.	7	11	1	10	-	4	1	-	27	17.50	1.54
	Oct.	8	6	5	18	1	2	-	-	32	24.00	1.33
	Nov.	7	2	6	5	-	-	-	-	13	20.25	0.64
	Dec.	1	-	-	-	-	-	-	-	-	1.50	0.00
Total		46	48	33	42	1	64	4	-	192	127.25	1.51
1955	Jan.	1	1	-	-	-	-	-	-	1	3.00	0.33
	May	3	-	2	1	-	1	-	-	4	9.25	0.43
	July	4	-	3	-	-	3	-	-	6	9.25	0.65
	Aug.	8	3	6	2	-	3	1	-	15	23.75	0.63
	Sept.	3	-	3	10	-	2	-	-	15	8.25	1.82
Total		19	4	14	13	-	9	1	-	41	53.50	0.77

local, i.e., confined to the area of fishing. This is suggested for skipjack, at least, by comparing our trolling results with those of the commercial pole-and-line fishery. Instead of a downward trend over the period 1951 to 1953, in the commercial fishery both 1951 and 1953 were years of high availability whereas 1952 was a year of low availability (Yamashita, In press). The comparison is vitiated in part by differences in size composition of the catches; the troll-caught fish were mostly small, whereas the commercially caught fish were a mixture of small and large sizes.

The average monthly availability of troll-catchable fish for the combined data of the years 1951 to 1955 is shown in figure 3. Although fish were caught in all months of the year except December (when effort was low), the highest availability occurred from May to October. Skipjack and dolphin were taken throughout most of the year but with a peaking of availability during the summer months. The commercial skipjack catch also peaks during the summer months (Yamashita, In press). No little tunny were taken during January to April, but this cannot be regarded as significant in view of the small number of hours trolled during this period. Welsh (1949b) reported good catches of this species during March, April, and May, 1948 and during February and April 1949. The peaking of the availability of yellowfin during September and/or October seems to be a common occurrence, for it was encountered during each of the three years (1951, 1954, and 1955) in which fishing was done during these months.

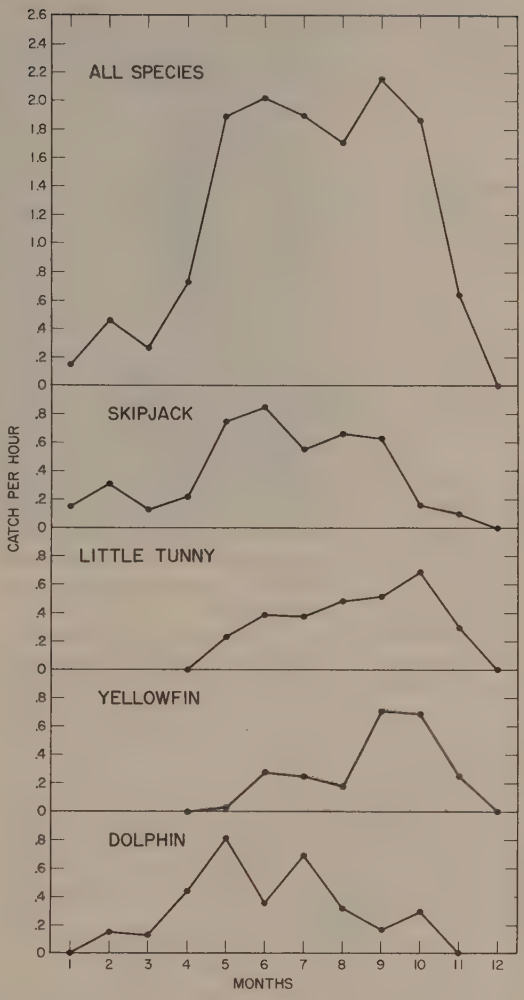

Figure 3.--Average monthly catch per hour's trolling for all species combined and for the four most abundant species in the catch over the period 1951 to 1955.

LURE PREFERENCE

During 1951 and 1953 records were kept of the strikes and catches on the several lures which were used. The lures, as shown in figure 4, were of three general types: (1) "metal squid" with a heavy blue, silver, or red metal body and white rubber arms, (2) "feather" with a heavy white or red metal head or a hollow silver head to which feathers of variegated color are attached, and (3) "rubber squid," with brown pliable rubber body and arms. Other types used to a small extent included a "plastic squid" and a "rubber fish."

Data on catch rate for all species of fish are shown in table 2.[2/] By comparing the strikes per lure per hour with the catch per lure per hour, it is at once apparent that about the same proportion of fish was lost from each lure during hauling of the line. Of the 3 lures used most extensively in 1951, the brown rubber squid produced the highest catch rate (0.61 fish per lure per hour), the metal squid with red body the second highest (0.42), followed by the feather lure with white head (0.29). The same order persisted when the data were selected to include only days in which the three lures were used simultaneously, with the following catch rates: 0.66, 0.44, and 0.30. However, in 1953

[2/] The fish catch for 1953 does not agree with that of table 1 because lure data were not available for a few days of that year.

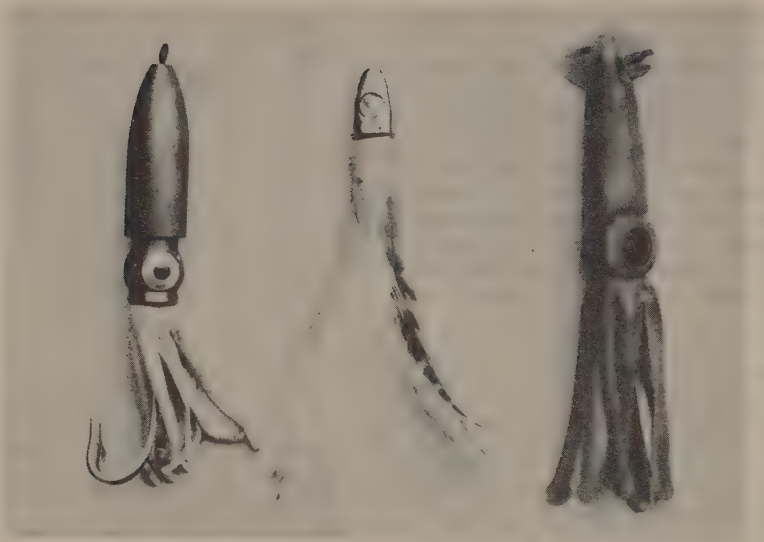

Figure 4.--The three main types of lures used in trolling.

Table 2.--Catch rate for the several lures used in 1951 and 1953

Year	Lure	Hours trolled	Number of strikes	Number of fish caught	Strikes per lure per hour	Catch per lure per hour
1951	Feather: white head	105-1/2	41	31	0.39	0.29
	Feather: red head	13	8	8	0.62	0.62
	Feather: hollow head	43-3/4	25	18	0.57	0.41
	Metal squid: blue body and white rubber arms - "A"	40-3/4	22	19	0.54	0.47
	Metal squid: silver body and white rubber arms - "B"	50-1/4	27	19	0.54	0.38
	Metal squid: red body and white rubber arms - "D"	288	148	121	0.51	0.42
	Metal squid: - "L"	29-1/2	12	8	0.41	0.27
	Metal squid: - "Y"	5-1/4	1	-	0.19	-
	Rubber squid: brown body and arms - "S"	283	213	174	0.75	0.61
	Plastic squid: transparent body and arms - "Z"	33	8	7	0.24	0.21
	Rubber fish: green	9-3/4	4	4	0.41	0.41
1953	Feather: white head	36-1/4	17	13	0.47	0.36
	Metal squid: blue body and white rubber arms - "A"	2-1/2	1	1	0.44	0.44
	Metal squid: red body and white rubber arms - "D"	61-1/4	22	17	0.36	0.28
	Metal squid: "Lai" (Scomberoides sp.) skin skirt	20-1/2	1	1	0.05	0.05
	Rubber squid: brown body and arms - "S"	95-1/2	42	33	0.44	0.35

neither the gross nor the selected data showed the same order. Ranged as above, i.e., brown rubber squid, metal squid with red body, and feather lure, the catch rates were as follows: 0.35, 0.28, and 0.36 for gross data (table 2) and 0.26, 0.18, and 0.26 for selected data. There is no clear evidence that one lure was more attractive than another with respect to form. Similarly there is no significant indication that red lures were more attractive than those of other color, as suggested by Welsh (1949b).

The number of fish caught according to species and lure is shown in table 3 for the four more important species and the three most used lures. In the 1951 data there is the suggestion of a differential attraction; for example, the rubber squid seemed to catch relatively more yellowfin than the other lures. However, the interaction chi-square for the 4 x 3 contingency table is not significant. Moreover, the rather meager 1953 data give no hint of lure preference. Apparently all three lures were about equally attractive to all four species.

SIZE COMPOSITION

The weight distributions of troll-caught skipjack, little tunny, yellowfin, and dolphin are given respectively in tables 4 to 7 and will be discussed below. Formulae for the conversion of weight to length will be given in a later section.

The weight distribution of the remainder of the catch is dismissed with the following notations: 23 frigate mackerel averaged 1-1/2 pounds and ranged from 1 to 2-1/2 pounds; 9 wahoo averaged 26 pounds and ranged from 20 to 33-1/2 pounds; 2 jacks weighed 4 and 8 pounds.

Skipjack

During the 5-year period 288 skipjack (excluding pole-and-line-caught fish) were measured. They averaged 4.9 pounds (47.5 cm.) and ranged from 2 to 17 pounds (30 to 70 cm.) (table 4).

From the monthly weight frequency histograms shown in figure 5 for the combined data of all years, it is evident that the majority of the fish ranged from 3 to 6 pounds with a pronounced mode at 4 pounds. Modal groups of similar size (varying from 44 to 50 cm., i.e., 3-1/2 to 6 pounds) were noted in the commercial catch by Brock (1954), and were considered most likely to be 1-year-olds, i.e., fish near the end of their first or the beginning of their second year of life. As Brock's data indicate that spawning extends over a 7-month period (February to September), a considerable size range of the "1-year-olds" would be expected. Presumably the majority of the troll-caught fish may be placed in this age category.

In figure 5 there is no apparent progression of the modal groups from month to month. This indicates that as the fish grow larger, they no longer frequent the inshore habitat that was fished. That they occur offshore is shown by modal groups ranging from 3-1/2 to 29 pounds (44 to 81 cm.) in samples from the commercial fishery examined by Brock (1954).

Table 3.--Number of fish of the more important species taken by each of the three most used lures

Year	Lure	Skipjack	Little tunny	Yellowfin	Dolphin	Total
1951	Feather: white head	12	10	3	5	30
	Metal squid: red body and white arms	34	31	9	34	108
	Rubber squid: brown body and arms	63	37	28	38	166
	Total	109	78	40	77	304
1953	Feather: white head	4	2	2	5	13
	Metal squid: red body and white arms	5	4	3	5	17
	Rubber squid: brown body and arms	11	6	5	11	33
	Total	20	12	10	21	63

Table 4.--Size frequency of troll-caught skipjack (1951-55)

Year	Month	Pounds																	Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
1951	Feb.	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	2
	Mar.	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	Apr.	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	2
	May	-	-	-	10	3	7	1	1	-	-	-	-	-	-	-	-	-	22
	June	-	-	4	12	10	7	2	1	1	-	1	-	-	-	-	-	-	38
	July	-	-	5	6	6	3	2	1	1	-	-	-	-	-	-	-	-	24
	Aug.	-	-	1	19	10	5	2	-	1	-	-	-	-	-	-	-	-	38
	Sept.	-	-	2	13	1	3	-	-	-	-	-	-	-	-	-	-	-	19
	Oct.	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	2
Total		-	-	12	60	32	29	7	4	3	-	1	-	-	-	-	-	-	148
1952	June	-	-	1	10	6	3	-	-	-	-	-	1	-	-	-	-	-	21
	July	-	-	1	17	4	4	2	-	-	-	-	-	-	-	-	-	-	29
	Aug.	-	-	2	8	1	3	-	-	-	-	-	-	-	-	-	-	-	12
Total		-	-	3	35	11	10	2	-	-	-	-	1	-	-	-	-	-	62
1953	June	-	-	7	10	1	-	-	-	-	-	-	-	-	-	-	-	-	18
	July	-	-	1	-	1	2	-	-	-	-	-	-	-	-	-	-	-	4
	Aug.	-	-	2	1	2	-	-	-	-	-	-	-	-	-	-	-	-	5
Total		-	-	10	11	4	2	-	-	-	-	-	-	-	-	-	-	-	27
1954	June	-	-	-	-	4	-	-	1	-	-	-	-	-	-	-	-	-	5
	July	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	3
	Aug.	-	-	2	5	-	7	3	2	-	2	-	-	-	-	-	-	-	21
	Sept.	-	-	-	4	3	1	-	1	1	1	-	-	-	-	-	-	-	11
	Oct.	-	1	2	2	-	-	1	-	-	-	-	-	-	-	-	-	-	6
	Nov.	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total		-	3	4	13	8	8	4	4	1	3	-	-	-	-	-	-	-	48
1955	Jan.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	Aug.	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total		-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	1	3

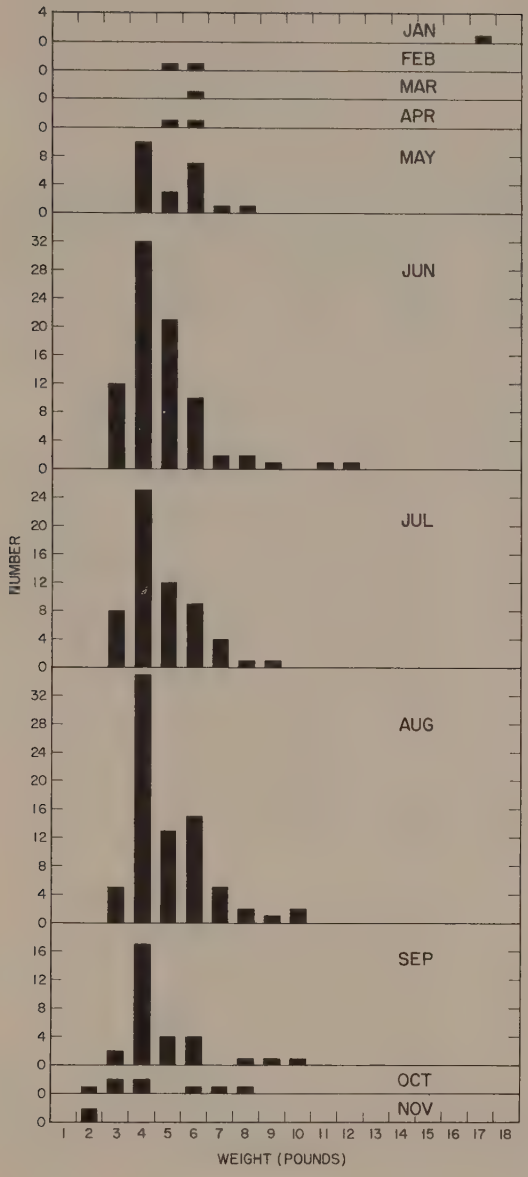

Figure 5.--Weight composition of skipjack by months for the combined data of 1951 to 1955.

Little Tunny

Measurements were made of 208 little tunny, which averaged 4.8 pounds (48 cm.) and ranged from about 1 to 15 pounds (30 to 70 cm.).

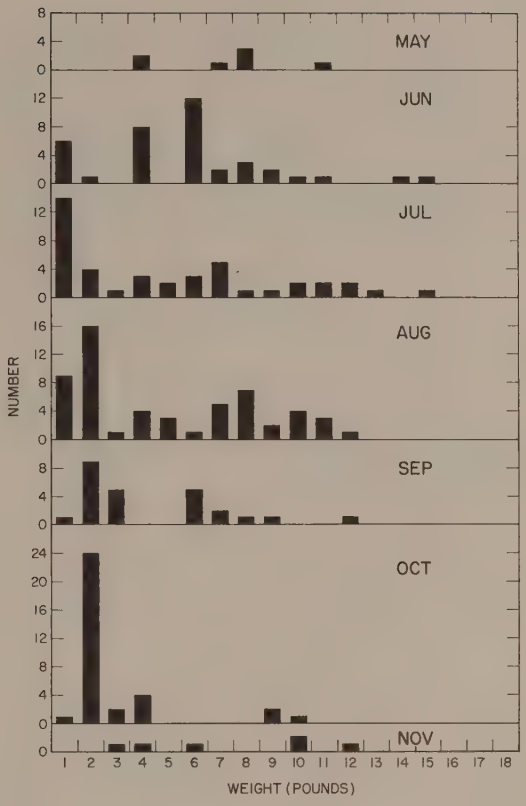

Figure 6.--Weight composition of little tunny by months for the combined data of 1951 to 1955.

The weight composition by months for the combined data of all years, shown in figure 6, suggests a slight progression of modes initially located at 1 and 6 pounds in June. However, the progression may be an artifact caused by combining variable annual data, for it is not indicated in the data for individual years (table 5). Rather, these data show a strong influx of 2-pound (36 cm.) fish during August, September, and October, 1951, and of 1-pound fish (30 cm.) during June, July, and August, 1952. There are relatively small numbers of 1- and 2-pound fish in other years. Fish of 6 to 8 pounds (50 to 57 cm.) seem to form a second modal group in 1951, 1954, and 1955, whereas a third modal group at 10 to 11 pounds (60 to 63 cm.) may be present in 1952. Thus the presence of at least two and possibly three age groups is indicated. Further discussion is not warranted because of the paucity of the present data.

Table 5.--Size frequency of troll-caught little tunny (1951-55)

Year	Month	Pounds																	Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1951	May	-	-	-	2	-	-	-	3	-	-	-	-	-	-	-	-	-	-	5
	June	-	-	-	-	-	6	1	1	-	-	-	-	-	-	1	-	-	-	9
	July	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	3
	Aug.	-	4	-	-	-	1	4	4	-	-	-	-	-	-	-	-	-	-	13
	Sept.	1	8	4	-	-	3	2	1	1	-	-	1	-	-	-	-	-	-	21
	Oct.	1	22	2	2	-	-	-	-	1	1	-	-	-	-	-	-	-	-	29
Total		2	34	6	4	-	10	10	9	2	1	-	1	-	-	1	-	-	-	80
1952	June	3	1	-	1	-	-	-	-	-	1	1	-	-	1	-	-	-	-	8
	July	13	3	1	2	-	2	-	1	-	2	2	2	1	-	-	-	-	-	29
	Aug.	9	11	-	2	3	-	-	-	-	2	1	1	-	-	-	-	-	-	29
Total		25	15	1	5	3	2	-	1	-	5	4	3	1	1	-	-	-	-	66
1953	June	2	-	-	4	-	2	-	-	-	-	-	-	-	-	-	-	-	-	8
	July	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	Aug.	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Total		3	1	1	6	2	2	-	-	-	-	-	-	-	-	-	-	-	-	15
1954	June	1	-	-	3	-	4	1	2	2	-	-	-	-	-	-	-	-	-	13
	July	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	3
	Aug.	-	-	-	-	-	-	-	1	1	1	2	-	-	-	-	-	-	-	5
	Sept.	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
	Oct.	-	2	-	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	5
	Nov.	-	-	1	1	-	1	-	-	-	2	-	1	-	-	-	-	-	-	6
Total		1	2	1	6	-	7	3	3	4	3	2	1	-	-	-	-	-	-	33
1955	May	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	2
	July	-	1	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	3
	Aug.	-	-	-	1	-	-	1	2	1	1	-	-	-	-	-	-	-	-	6
	Sept.	-	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	3
Total		-	2	1	1	-	1	2	2	2	1	1	-	-	-	1	-	-	-	14

Yellowfin

Measurements were made on 147 yellowfin, which averaged 6.5 pounds (54 cm.) and ranged from 1 to 18 pounds (29 to 72 cm.) (table 6).

The monthly data shown in figure 7 indicate the progression of a mode starting at about 5 or 6 pounds in June and advancing to about 10 pounds in November. This apparent growth agrees well with that observed for a yellowfin held in captivity, which increased in weight from about 5 pounds when it started feeding on July 2, 1951, to 11 pounds when it died on January 13, 1952 (Tester 1952).

Based on a study of the ovaries, June (1953) concludes that yellowfin in Hawaiian waters spawn from the middle of May to the end of October. Moore (1951), from a study of the weight composition of the commercial pole-and-line and longline catches, concludes that yellowfin attain an average weight of about 7 pounds (54 cm.) by the end of their first year and an average weight of 46 pounds (103 cm.) by the end of their second year of life. Our data agree well with Moore's estimate of the weight at the end of the first year, i.e., the mean weight of our troll-caught fish is 6.8 pounds during August, assuming this month to be the middle of the spawning period. However, fish which average 7 pounds in August and 10 pounds in November would have to grow very rapidly during the winter, spring, and early summer to attain a mean weight of 46 pounds during the following August. It may be that our estimate of 10 pounds in November is biased by selective sampling, i.e., the fish may tend to move out of the area as they get larger, although the advancing mode noted above seems to indicate the contrary.

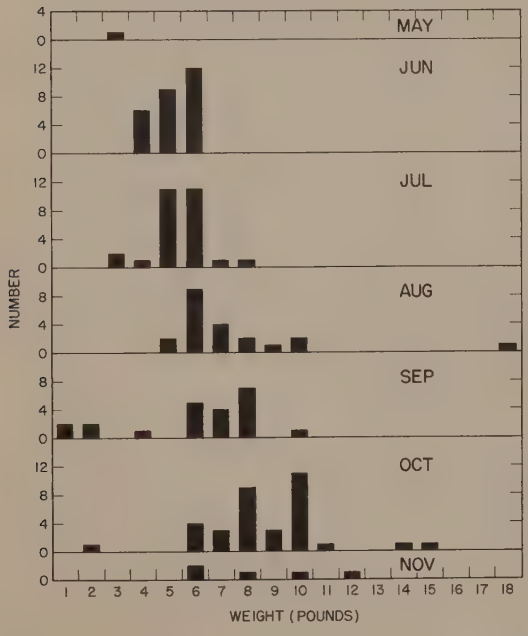

Figure 7.--Weight composition of yellowfin by months for the combined data of 1951 to 1955.

Dolphin

Measurements were made on 198 dolphin which averaged 6.8 pounds (72 cm.) and ranged from 1-1/2 to 37 pounds (41 to 121 cm.) (table 7).

From the monthly weight distribution, shown in figure 8, there is some indication of bimodality

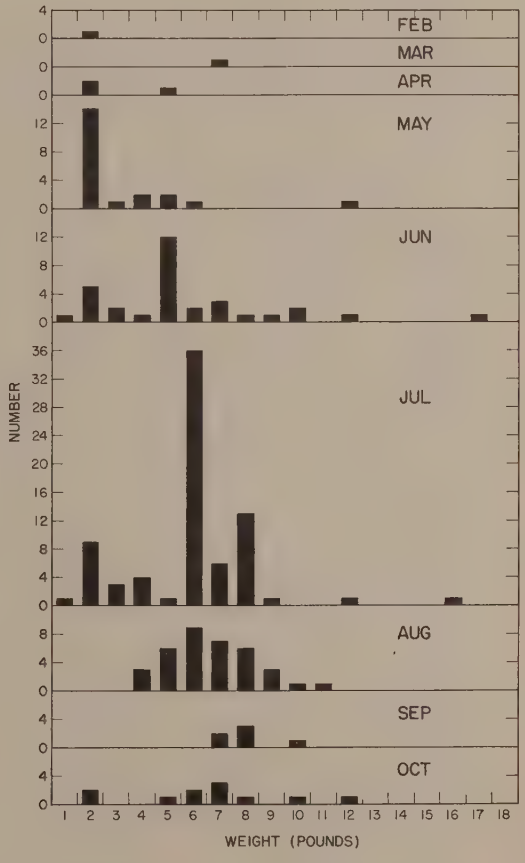

Figure 8.--Weight composition of dolphin by months for the combined data of 1951 to 1955.

Table 6.--Size frequency of troll-caught yellowfin (1951-55)

Year	Month	Pounds																	Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1951	June	-	-	-	-	9	5	-	-	-	-	-	-	-	-	-	-	-	-	14
	July	-	-	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	4
	Aug.	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	3
	Sept.	2	2	-	-	-	2	2	6	-	-	-	-	-	-	-	-	-	-	14
	Oct.	-	1	-	-	-	2	-	6	1	5	-	-	-	-	-	-	-	-	15
Total		2	3	2	-	9	13	3	12	1	5	-	-	-	-	-	-	-	-	50
1952	June	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
	July	-	-	-	1	10	8	-	-	-	-	-	-	-	-	-	-	-	-	19
	Aug.	-	-	-	-	2	3	1	-	-	2	-	-	-	-	-	-	-	-	8
	Oct.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Total		-	-	-	7	12	11	1	-	-	3	-	-	-	-	-	-	-	-	34
1953	June	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	7
	July	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	3
Total		-	-	-	-	1	7	1	1	-	-	-	-	-	-	-	-	-	-	10
1954	July	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
	Aug.	-	-	-	-	-	2	2	2	1	-	-	-	-	-	-	-	-	1	8
	Sept.	-	-	-	1	-	3	2	1	-	1	-	-	-	-	-	-	-	-	8
	Oct.	-	-	-	-	-	2	3	3	2	5	1	-	-	1	1	-	-	-	18
	Nov.	-	-	-	-	-	2	-	1	-	1	-	1	-	-	-	-	-	-	5
Total		-	-	-	1	-	10	7	7	3	7	1	1	-	1	1	-	-	1	40
1955	May	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Aug.	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2
	Sept.	-	-	-	-	-	4	4	-	-	-	2	-	-	-	-	-	-	-	10
Total		-	-	1	-	-	6	4	-	-	-	2	-	-	-	-	-	-	-	13

Table 7.--Size frequency of troll-caught dolphin (1951-55)

Year	Month	Pounds																																			Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
1951	Feb.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Mar.	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Apr.	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	May	-	14	1	2	2	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	23
	June	-	-	-	1	10	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
	July	-	1	-	1	1	6	1	5	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
	Aug.	-	-	-	-	4	5	5	5	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21
	Sept.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	2
	Oct.	-	-	-	-	1	2	3	1	-	1	-	1	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12
Total		-	18	1	4	19	14	12	11	1	2	-	3	-	-	-	-	-	-	-	1	1	1	-	1	-	1	-	-	1	-	-	1	-	-	-	-	-	92
1952	June	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	5
	Aug.	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total		-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	6
1953	June	1	1	2	-	2	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
	July	-	3	3	3	-	5	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
	Aug.	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total		1	4	5	3	2	7	1	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
1954	June	-	4	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
	July	1	5	-	-	-	23	5	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	41
	Aug.	-	-	-	2	1	4	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
	Sept.	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	Oct.	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total		1	11	-	2	1	27	7	10	2	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64
1955	May	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	July	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	Aug.	-	-	-	1	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	Sept.	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total		-	-	-	1	1	2	2	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9

in fish less than 10 pounds in weight, with one mode at 2 pounds (46 cm.) and a second ranging from 5 to 8 pounds (64 to 76 cm.). Only a few specimens larger than 10 pounds were taken over the period.

WEIGHT-LENGTH RELATIONS

Weight-length relations were calculated for each of the four most abundant species according to the usual formula relating weight, W (in pounds), with length, L (in centimeters):

$$W = aL^b$$

$$\log W = \log a + b \log L$$

The constants log a and b as determined by least squares are given in table 8, along with other pertinent data. Although the numbers of fish are small, the calculations are included because samples of small fish are usually unavailable from commercial catches.

With the dolphin, unlike the tunas, there was an indication of sexual dimorphism in the weight-length relation. Beyond 6 pounds (68 cm.) the males tended to be heavier for a given length than the females (fig. 9). This is probably related to a well-known sexual dimorphism in the shape of the head, the males having a much higher forehead than the females (Schuck 1951).

SEX AND MATURITY

Summary data on sex and gonad maturity of six species of fish are given in table 9. The numbers of fish do not agree with those of previous tables as many of the fish, particularly little tunny, yellowfin, and dolphin, were transferred alive to holding ponds and consequently were not examined in respect to sex or stage of maturity.

Skipjack

Of 347 troll- and pole-and-line-caught skipjack, 3 had gonads so undeveloped that the sex could not be determined. The remainder comprised 61.0 percent males and 39.0 percent females. The departure from a 1:1 ratio was statistically significant as determined by the chi-square test.

Brock (1954) points out that, in his samples from the commercial catch, males were significantly more abundant than females in the fall of the year (September to December) but not in spring (March to May) nor in the summer (June to August). When our data are arranged according to season (table 10) there is a significant preponderance of males in the summer. At present we can offer no satisfactory explanation of these differences in sex ratio.

Of the 344 fish which could be sexed, 12 were recorded as "immature" (gonad stage 1) and 4 were "spent" (stage 5). Some of the fish recorded as "immature" may have been spent, for they ranged in weight from 3 to 17 pounds. The four fish classed as "spent" ranged from 5 to 7 pounds. Despite the apparent confusion in separating these two stages, it is evident (table 9) that the majority of the fish were "maturing" or "nearly mature" and that a few may have spawned. Considering their size and assumed age, it seems safe to conclude with Brock that skipjack may mature in 1 year.

Little Tunny

It was impossible to determine the sex of 8 small (1 to 2 pounds) little tunny. Of the remaining 93 fish, males were slightly but not significantly less numerous than females, forming 48.4 percent of the catch.

The gonads of most of the fish were in the "maturing" condition (stages 2 and 3). The 7

Table 8.--Number of fish, size range, and constants in the weight-length relations (log W = log a + b log L) for four species of troll-caught fish

Species	Number of fish	Size range (pounds)	Log a	b
Skipjack	268	2-1/2 to 12-1/2	-4.7262	3.2164
Little tunny	101	1 to 15	-4.6229	3.1544
Yellowfin	59	1 to 17-3/4	-4.2015	2.9045
Dolphin	133	1-1/2 to 12	-4.1966	2.7111

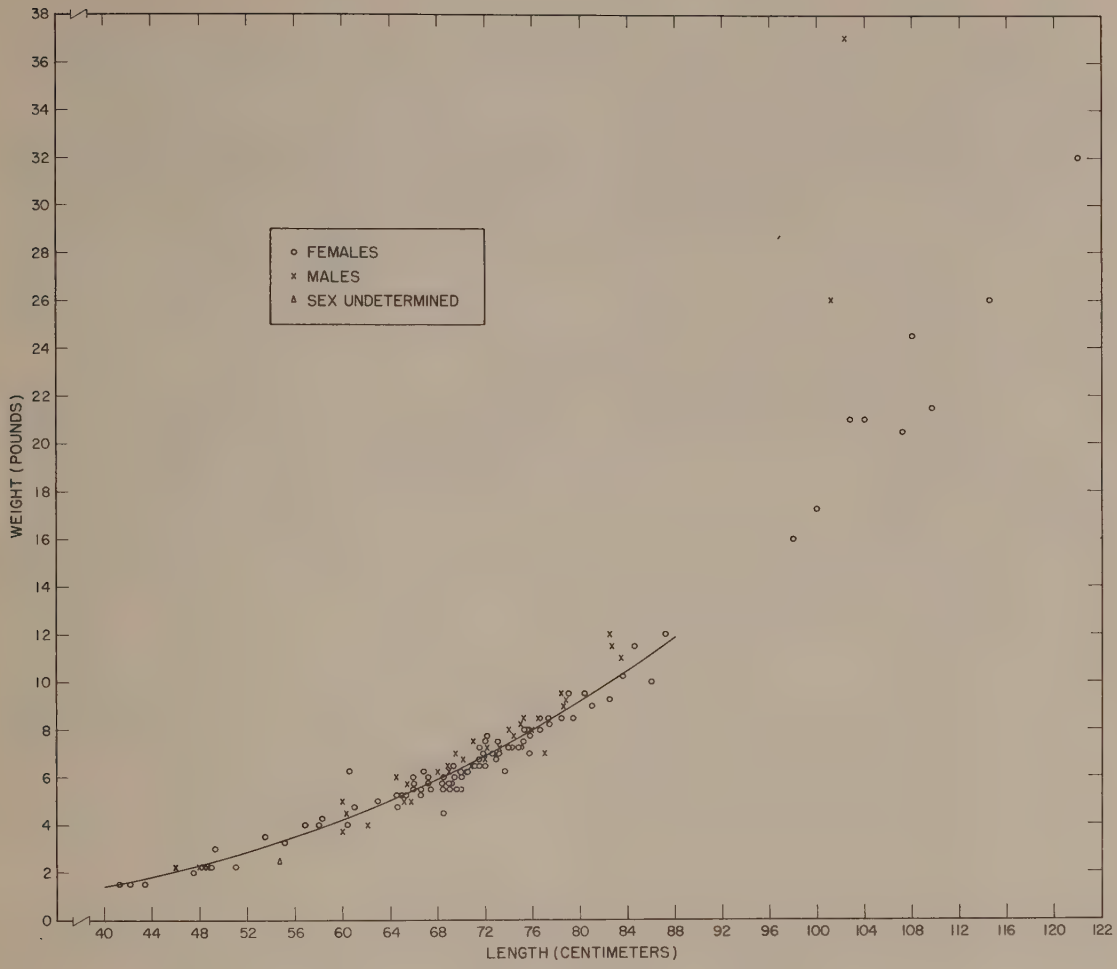

Figure 9.--Weight-length relation of dolphin.

fish classed as "spent" (stage 5) comprised 2 fish of about 5 pounds and 5 fish of 10 to 11 pounds.

Yellowfin

In contrast to skipjack and little tunny, most of the yellowfin were judged to be immature. Of the 59 fish examined for sex, 14 had gonads so immature that the sex could not be ascertained. The remainder were mostly "immature" (stage 1) with a few "maturing" (stage 2). In view of the size of these fish and their presumed age, it would seem doubtful that yellowfin mature by the end of their first year.

Of the yellowfin which could be sexed, males were in the minority (45 percent), although the ratio did not deviate significantly from 1:1. This is in agreement with the sex ratio of the smaller (less than 80 to 90 pounds) fish taken by longline near the Equator, but it is in contrast to the sex ratio for the larger longline-caught fish both near the Equator and in Hawaiian waters, in which males strongly predominate (Iverson 1956). The reason for the change in sex ratio is obscure.

Dolphin

The dolphin showed a significant deviation from the 1:1 ratio, with males (28.5 percent) much less numerous than females (71.5 percent). All males were either "immature" (stage 1) or in early stages of maturing (stage 2). The females included stages ranging from "immature" to "mature" (stage 4). The fish classed as "immature" ranged from 1-1/2 to 8-1/2 pounds. Of the 12 fish classed as "mature" (but not "running ripe")

Table 9.--Sex and maturity stage of the various species

Species	Sex	Maturity stage					
		1 (immature)	2 (maturing)	3 (nearly mature)	4 (ripe)	5 (spent)	All
Skipjack	Males	6	43	158	2	1	210
	Females	6	18	97	10	3	134
	Undetermined	-	-	-	-	-	(3)
	Total	12	61	255	12	4	344
Little tunny	Males	3	13	24	1	4	45
	Females	11	12	17	5	3	48
	Undetermined	-	-	-	-	-	(8)
	Total	14	25	41	6	7	93
Yellowfin	Males	18	2	-	-	-	20
	Females	19	6	-	-	-	25
	Undetermined	-	-	-	-	-	(14)
	Total	37	8	-	-	-	45
Dolphin	Males	18	24	-	-	-	42
	Females	36	20	37	12	-	105
	Total	54	44	37	12	-	147
Frigate mackerel	Males	1	3	2	1	-	7
	Females	-	4	6	-	-	10
	Total	1	7	8	1	-	17
Wahoo	Males	-	5	-	-	-	5
	Females	-	1	1	1	-	3
	Total	-	6	1	1	-	8

Table 10.--Number of male and female skipjack taken during four periods of the year and the probability that the sex ratio is not significantly different from 1 : 1 as determined by a chi-square test

Sex	December-February	March-May	June-August	September-November	Total
Male	0	12	174	24	210
Female	3	13	105	13	134
Both	3	25	279	37	344
Probability		>0.05	<0.01	>0.05	<0.01

7 ranged from 5 to 8 pounds and 5 ranged from 10 to 32 pounds.

There is considerable diversity in the proportion of males and females between schools. For example, 11 females and 6 males were caught from a school of 5- to 9-pound fish on August 8, 1951. In contrast, of 31 fish taken from a school of 5- to 9-pound fish on July 12, 1954, 29 were females and only 2 were males.

Other Fish

We have few data on frigate mackerel and wahoo. It might be noted, however, that the 17 frigate mackerel, which were all about 1-1/2 pounds in weight, were mostly at stages 2 and 3, but included one male (2-1/2 pounds) classed as "mature." The 8 wahoo, which ranged from 20 to 33 pounds, were mostly at stage 2, but the largest specimen, a female, was classed as "mature."

FOOD

The results of the stomach analyses for skipjack, little tunny, yellowfin, and dolphin are presented in tables 11, 12, 13, and 14, respectively. The numbers of stomachs examined were as follows: 67 skipjack, 32 little tunny, 15 yellowfin, 52 dolphin, 3 wahoo, and 2 frigate mackerel. The stomach collections were made in 1951, 1952, and 1953.

The advantages and disadvantages of the various methods of evaluating food components have been discussed by Reintjes and King (1953). They conclude "....that those food items that rank large in number, large in volume, and high in frequency of occurrence are important foods—at the time and in the area sampled." Data for all three of these methods of analysis have been incorporated into the above-mentioned tables.

In identifying the ingested fishes, the following references proved to be helpful: Brock (1950), Jordan and Evermann (1905), and Smith (1949). The scientific names of the food fishes referred to in this report are for the most part in accordance with Brock (1950). Identification of the invertebrates was aided by reference to Berry (1914), Edmondson (1946), Hiatt (no date), and Townsley (1950).

We wish to thank Drs. A. H. Banner and D. W. Strasburg for their help in the identification of various organisms found in the stomachs.

The food of these troll-caught fishes is composed almost entirely of fishes, cephalopods, and crustaceans. This is in agreement with the results of other investigators who have noted the stomach contents of these same pelagic species in Hawaii and elsewhere (Imamura 1949, Nakamura 1949, Reintjes and King 1953, Ronquillo 1953, Shapiro 1948, Welsh 1949a, and others). A discussion of the food of each species follows.

Skipjack

For skipjack ranging in size from 40.0 to 61.1 cm. the most important food item by volume appeared to be cephalopods (squid), with fishes second in importance (table 11). Not only did squid comprise the largest volume, but the numbers found were also high, lending further support to their importance. This is in contrast to results which were obtained by Welsh (1949a) from samples collected in waters adjacent to Oahu but not confined to the area delineated in figure 1. In 21 stomachs he found fishes to be the most important food by volume. In our samples fishes did have the highest frequency of occurrence.

Of the crustaceans the stomatopod larvae comprised the largest volume and highest occurrence. Some of the skipjack were found to be gorged with stomatopods. Some were also found to be gorged with crab megalops.

Little Tunny

The results for the little tunny ranging in size from 31.2 to 66.8 cm. show that fishes comprised the greatest volume of food with crustaceans next in rank (table 12). The 196 samples examined by Welsh (1949a) for this same species showed just the opposite.

Gorging on stomatopods and crab megalops was also observed for the little tunny.

Yellowfin

In the food of yellowfin ranging in size from 47.4 to 58.9 cm., squid provided the greatest bulk (table 13). Welsh (1949a) also found that squid were important as food for yellowfin around the island of Oahu. Reintjes and King (1953) found that cephalopods were relatively unimportant in the food of yellowfin caught at the surface near land in the region of the Line and Phoenix islands.

The presence of seahorses (Syngnathidae) in the stomach contents of yellowfin in Hawaiian waters has been previously reported by Herald (1949). As seen in table 13, seahorses were

Table 11.--Stomach contents of 67 troll-caught skipjack (number of stomachs: 49 in 1951, 10 in 1952, 8 in 1953)

Food organisms	Total volume		Stomachs in which occurred		Number of organisms
	cm.^{3}	Percent	Number	Percent	
Fishes					
Exocoetidae					
Parexocoetus brachypterus	0.7	---*	1	1.5	1
Sphyraenidae					
Sphyraena helleri	6.0	0.2	1	1.5	1
Serranidae					
Unidentified sp.	13.3	0.5	2	3.0	14
Bramidae					
Collybus drachme	166.2	6.3	7	10.4	33
Carangidae					
Unidentified sp.	264.0	10.0	10	14.9	18
Chaetodontidae					
Unidentified sp.	2.3	---*	1	1.5	4
Acanthuridae					
Unidentified sp.	7.1	0.3	2	3.0	3
Unidentified fish remains	225.5	8.6	32	47.8	88
Total fishes	685.1	26.0			
Cephalopoda					
Decapoda					
Ommastrephidae					
Ommastrephes hawaiiensis	810.6	30.8	13	19.4	41
Unidentified sp.	594.9	22.6	12	17.9	33
Sepiolidae					
Heteroteuthis hawaiiensis	247.4	9.4	4	6.0	Numerous
Unidentified squid	116.0	4.4	11	16.4	22
Total decapod cephalopods	1768.9	67.2			
Crustacea					
Stomatopoda					
Unidentified larvae	161.4	6.1	25	37.3	Numerous
Decapoda					
Unidentified shrimp	0.1	---*	1	1.5	7
Unidentified anomuran	0.1	---*	1	1.5	1
Brachyuran megalopa	15.1	0.6	20	30.0	Numerous
Total crustaceans	176.7	6.7			
Grand total	2630.7				

* Less than 0.1 percent.

Table 12.--Stomach contents of 32 troll-caught little tunny (number of stomachs: 20 in 1951, 11 in 1952, 1 in 1953)

Food organisms	Total volume		Stomachs in which occurred		Number of organisms
	$cm.^3$	Percent	Number	Percent	
Fishes					
Syngnathidae					
Hippocampus sp.	1.0	---*	2	6.2	2
Serranidae					
Unidentified sp.	516.3	28.3	8	25.0	Numerous
Priacanthidae					
Priacanthus cruentatus	26.2	1.4	1	3.1	1
Unidentified sp.	121.2	6.6	1	3.1	8
Bramidae					
Collybus drachme	30.3	1.7	1	3.1	1
Carangidae					
Decapturus sp.	308.0	16.9	2	6.2	Numerous
Unidentified sp.	151.2	8.3	3	9.4	6
Ostraciidae					
Lactoria diaphanus	2.3	0.1	3	9.4	6
Pegasidae					
Pegasus papilio	0.4	---*	1	3.1	1
Unidentified fish remains	518.1	28.4	20	62.5	Numerous
Total fishes	1675.0	91.8			
Cephalopoda					
Decapoda					
Unidentified squid	1.3	---*	1	3.1	1
Total decapod cephalopods	1.3	---*			
Crustacea					
Stomatopoda					
Unidentified larvae	28.4	1.6	7	21.9	Numerous
Decapoda					
Unidentified caridean shrimp	101.0	5.5	2	6.2	Numerous
Penaeidae					
Unidentified sp.	1.0	---*	1	3.1	1
Unidentified reptantian larvae	1.8	---*	1	3.1	4
Brachyuran megalopa	16.6	0.9	5	15.6	Numerous
Total crustaceans	148.8	8.2			
Grand total	1825.1				

* Less than 0.1 percent.

Table 13.--Stomach contents of 15 troll-caught yellowfin (number of stomachs: 10 in 1951, 4 in 1952, 1 in 1953)

Food organisms	Total volume		Stomachs in which occurred		Number of organisms
	cm.^{3}	Percent	Number	Percent	
Fishes					
Syngnathidae					
Hippocampus histrix	11.3	1.6	3	20.0	10
Hippocampus sp.	29.4	4.2	5	33.3	Numerous
Bramidae					
Unidentified sp.	86.5	12.4	1	6.7	5
Blennidae					
Runula sp.	0.4	---*	1	6.7	1
Dactylopteridae					
Dactyloptena orientalis	2.3	0.3	1	6.7	1
Balistidae					
Balistapus sp.	3.5	0.5	1	6.7	1
Monacanthidae					
Unidentified sp.	4.6	0.7	1	6.7	1
Ostraciidae					
Lactoria diaphanus	9.6	1.4	3	20.0	10
Lactoria sp.	0.4	---*	1	6.7	1
Tetrodontidae					
Arothron sp.	0.2	---*	1	6.7	1
Pegasidae					
Pegasus papilio	10.9	1.6	4	26.7	14
Unidentified fish remains	40.5	5.8	9	60.0	Numerous
Total fishes	199.6	28.6			
Cephalopoda					
Decapoda					
Ommastrephidae					
Ommastrephes hawaiiensis	380.1	54.4	4	26.7	9
Unidentified squid	48.8	7.0	6	40.0	Numerous
Total decapod cephalopods	428.9	61.4			
Crustacea					
Stomatopoda					
Unidentified larvae	59.7	8.6	15	100.0	Numerous
Euphausiacea					
Euphausidae					
Unidentified sp.	0.4	---*	1	6.7	1
Decapoda					
Unidentified caridean shrimp	1.0	0.1	1	6.7	3
Palinuridae					
Panulirus japonicus	1.0	0.1	1	6.7	1
Brachyuran megalopa	7.6	1.1	6	40.0	26
Total crustaceans	69.7	10.0			
Grand total	698.2				

* Less than 0.1 percent.

the most common identifiable group of the food fishes utilized by the yellowfin in our samples.

Dolphin

An examination of the stomachs of 52 dolphin ranging in size from 41.3 to 121.0 cm. showed the importance of fish in their diet (table 14). Cephalopods and crustaceans together comprised less than 3 percent of the total volume. Flyingfishes (Exocoetidae) appeared to be particularly significant both in volume and occurrence. Welsh's study of 58 stomach samples showed almost identical results. Flyingfish have also been reported in the food of dolphin caught in North Carolina waters (Schuck 1951).

Cymothoid isopods were found in two stomachs. Members of this family are known to be parasitic on fish. It is possible that they were parasitic on the food fishes and thus incidentally ingested. One of the stomachs containing cymothoid isopods also contained a monacanthid, an exocoetid, and unidentified fish remains. The other contained exocoetids and a syngnathid in addition to the isopod.

A further item of interest was the presence of a coral rock about 1/2 $cm.^3$ in volume in one of the stomachs. The rock may have been ingested accidentally while feeding on reef-dwelling forms. Other contents of this stomach were penaeid shrimps, stomatopod larvae, a crab megalopa, remains of a fish, and remains of a squid.

Wahoo

All three of the stomachs examined contained remains of fish. One of the food fishes was identified as a carangid. The sizes of these three wahoo were 104.1, 111.1, and ca. 123 cm.

Frigate Mackerel

Of the two frigate mackerel stomachs examined, one contained numerous crab megalops and stomatopod larvae, while the other contained 8 crab megalops, numerous stomatopod larvae, 2 anomuran larvae, and a small octopus. The frigate mackerel were 33.2 and 36.6 cm. in length.

General Discussion

The relative importance by volume of the three major food categories for the skipjack, little tunny, yellowfin, and dolphin is portrayed in figure 10. The little tunny and dolphin appear to rely heavily upon fishes for food, while the skipjack and yellowfin have a more varied diet utilizing all three of the major food groups.

The similarity between the diets of skipjack and yellowfin and between little tunny and dolphin suggests the likelihood of interspecific competition. Evidence of competition for food between skipjack and yellowfin was furnished on several occasions when the Salpa would catch both species while trolling under a working bird flock. The occurrence of the same invertebrate organisms in the stomachs of both skipjack and yellowfin seems to attest the presence of competition, but the absence of food fish species common to both fails to support it (tables 11 and 13). Evidence for competition between the little tunny and the dolphin may be seen in the number of food fish species common to both (tables 12 and 14). The fishes most prevalent in the food of the dolphin, however, were the flyingfishes

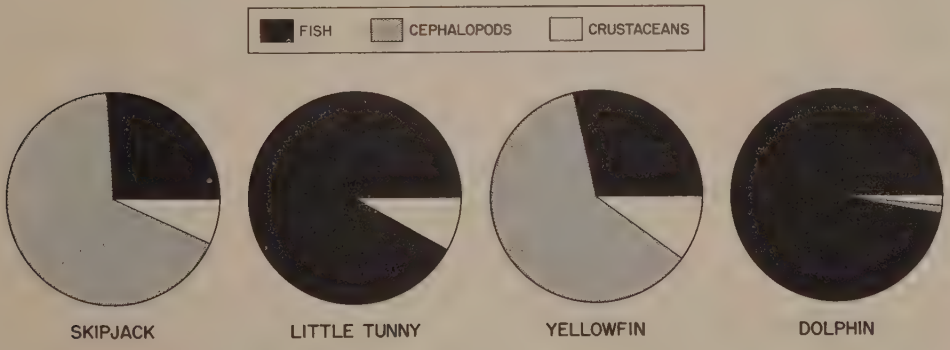

Figure 10.--Relative importance of fishes, cephalopods, and crustaceans in the diets of skipjack, little tunny, yellowfin, and dolphin.

Table 14.--Stomach contents of 52 troll-caught dolphin (number of stomachs: 53 in 1951, 5 in 1952, 14 in 1953)

Food organisms	Total volume		Stomachs in which occurred		Number of organisms
	cm.^{3}	Percent	Number	Percent	
Fishes					
Clupeidae					
Spratelloides delicatulus	3.4	---*	4	7.7	6
Synodontidae					
Synodus variegatus	0.5	---*	1	1.9	1
Belonidae					
Ablennes hians	121.0	3.4	1	1.9	1
Hemiramphidae					
Hemiramphus brasiliensis	239.2	6.7	2	3.8	2
Euleptorhamphus viridis	40.5	1.1	1	1.9	1
Exocoetidae					
Parexocoetus brachypterus	402.9	11.3	3	5.8	11
Exocoetus volitans	4.9	0.1	1	1.9	4
Cypselurus sp.	429.6	12.0	3	5.8	4
Unidentified sp.	605.8	17.0	16	30.8	Numerous
Syngnathidae					
Hippocampus histrix	8.3	0.2	4	7.7	7
Hippocampus sp.	9.0	0.3	4	7.7	8
Holocentridae					
Holocentrus sp.	3.5	---*	1	1.9	1
Serranidae					
Unidentified sp.	1.6	---*	1	1.9	1
Coryphaenidae					
Coryphaena hippurus	7.8	0.2	2	3.8	2
Mullidae					
Unidentified sp.	61.0	1.7	2	3.8	3
Carangidae					
Decapturus pinnulatus	14.4	0.4	1	1.9	1
Caranx melampygus	14.4	0.4	1	1.9	2
Unidentified sp.	32.3	0.9	2	3.8	2
Kyphosidae					
Kyphosus sp.	23.5	0.7	1	1.9	1
Chaetodontidae					
Chaetodon sp.	1.6	---*	1	1.9	4
Unidentified sp.	0.5	---*	1	1.9	1
Blenniidae					
Runula sp.	2.4	---*	4	7.7	5
Zanclidae					
Zanclus canescens	30.8	0.9	1	1.9	1
Istiophoridae					
Istiophorus orientalis	17.4	0.5	1	1.9	1
Scorpaenidae					
Taenianotus triacanthus	2.4	---*	1	1.9	1
Unidentified sp.	4.6	0.1	1	1.9	3
Scombridae					
Unidentified sp.	765.0	21.4	2	3.8	2

* Less than 0.1 percent.

Table 14.--Stomach contents of 52 troll-caught dolphin (number of stomachs: 53 in 1951, 5 in 1952, 14 in 1953) (cont'd)

Food organisms	Total volume		Stomachs in which occurred		Number of organisms
	cm.^{3}	Percent	Number	Percent	
Monacanthidae					
Cantherines pardalis	10.1	0.3	2	3.8	2
Cantherines sp.	5.4	0.2	1	1.9	1
Unidentified sp.	30.4	0.9	3	5.8	4
Ostraciidae					
Lactoria diaphanus	20.5	0.6	11	21.2	18
Ostracion lentiginosum	11.3	0.3	3	5.8	3
Tetrodontidae					
Unidentified sp.	30.7	0.9	2	3.8	2
Diodontidae					
Diodon holacanthus	46.1	1.3	3	5.8	8
Pegasidae					
Pegasus papilio	1.1	---*	1	1.9	1
Unidentified fish remains	470.2	13.2	25	48.1	Numerous
Total fishes	3474.1	97.3			
Cephalopoda					
Decapoda					
Unidentified squid	40.1	1.1	4	7.7	6
Total decapod cephalopods	40.1	1.1			
Crustacea					
Isopoda					
Cymothoidae					
Unidentified sp.	3.5	---*	2	3.8	3
Stomatopoda					
Unidentified larvae	45.3	1.3	16	30.8	Numerous
Decapoda					
Penaeidae					
Unidentified sp.	5.1	0.1	5	9.6	24
Brachyuran megalopa	4.0	0.1	6	11.5	12
Total crustaceans	57.9	1.6			
Grand total	3572.1				

* Less than 0.1 percent.

(Exocoetidae), which were not found in the stomachs of the little tunny. In view of the lack of specificity in the diets, it is probable that if all four species were present in the same area at the same time, they would vie with each other for whatever food was available.

Reliance upon inshore fauna was evident in the stomach contents of all four species, but this is not surprising since all of our catches were made relatively close to land. The pelagic larval and juvenile stages of many of the food fishes were those of species which spend their adult stages among the reefs or waters close to the reefs. This holds true for some of the invertebrates also. Undoubtedly the reefs of Kaneohe Bay and adjacent areas play an important role in contributing food to the area sampled. However, the extent of this contribution is presently unknown.

SUMMARY

1. Catch, catch rate, lure preference, size frequencies, weight-length relations, sex and maturity, and food are reported for tunas and other pelagic fishes caught by trolling off Kaneohe Bay, Oahu. Trolling operations were conducted aboard the University of Hawaii's research vessel Salpa during 1951 through 1955.

2. The bulk of the catch was composed of skipjack, dolphin, little tunny, and yellowfin. The highest availability for all species combined occurred in the months of May to October. Yellowfin availability was consistently high during September and October.

3. Among the various types of lures used, no preference was found with respect to form or color of the lure.

4. Weight distribution of skipjack showed no progression of modes, indicating migration away from the fishing area as the fish grew larger. The majority of the skipjack were presumed to be "1-year-olds." Weight distribution of little tunny appeared to indicate the presence of two and possibly three age groups. Weight distribution of yellowfin indicated growth from 5 or 6 pounds in June to 10 pounds in November. Weight distribution of dolphin appeared to indicate bimodality in fish less than 10 pounds.

5. Weight-length relations were calculated for skipjack, little tunny, yellowfin, and dolphin. Beyond 68 cm. male dolphin tended to be heavier than females at a given length.

6. The sex ratio of skipjack showed a preponderance of males. Gonad examinations indicate that skipjack may mature in 1 year. The sex ratio of little tunny showed approximately an equal proportion of males and females. Gonad examinations showed the majority of little tunny in the "maturing" stages, with a few mature and spent fish. The sex ratio for yellowfin was similar to that of the little tunny. Gonad examinations revealed no mature yellowfin. The sex ratio of dolphin showed a preponderance of females, although there was considerable diversity in the sex ratio between schools. Gonad examinations revealed the presence of mature females but no mature males.

7. Food analyses showed that the diet of these pelagic fishes comprised primarily fishes, cephalopods, and crustaceans. Some evidence of interspecific competition for food was indicated. Reliance upon inshore fauna was evident in the stomach contents of all the species examined.

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Contributions to the Knowledge of the Alpheid Shrimp of the Pacific Ocean
Part III. On a Small Collection from Onotoa, Gilbert Islands[1]

ALBERT H. BANNER[2]

THIS PAPER reports on a small collection of snapping shrimp made at Onotoa in the southern Gilbert Islands during the summer of 1951 by the members of the Coral Atoll Research team during the investigation of the total ecology of the atoll under the sponsorship of the Pacific Science Board with funds from the Office of Naval Research (Contract NR 160–165). Members of the team working on the ecology of marine animals were Dr. Preston E. Cloud, Jr. of the U. S. Geological Service, Dr. Donald W. Strasburg, Dr. John E. Randall, and myself. The latter three at the time were with the University of Hawaii.

The study of these shrimp was initiated under a grant administered by the U. S. National Museum and the Pacific Science Board under a contract between the Office of Naval Research, Biology Branch, and the National Academy of Sciences (NR 160–175); some help in the final phases of the study was gained through a grant from the National Science Foundation (NSF-G-1754). I also wish to acknowledge the aid that was given me in the field by Drs. Cloud, Strasburg, and Randall.

In order to save space and time in this paper, as in the second study, instead of giving complete synonymy and bibliographic references for each species, reference will be made to the earlier papers of the series whenever a species has been listed before.

Type specimens of the new species described in this paper will be deposited in the institution from which they were borrowed; those types in my personal collection will be deposited either in the U. S. National Museum or the Bernice P. Bishop Museum.

ONOTOA ATOLL

Onotoa is a small, relatively dry atoll in the southern portion of the Gilbert Islands. This portion is known also as the Kingsmill Islands. It lies at 1°47′ S., 175°29′ E. (north anchorage) and is slightly less than 12 miles long and 5 miles broad, while the land area, reaching along the eastern side of the lagoon, is only slightly more than half a mile wide at the broadest spot (Fig. 1). The windward or eastern side of the atoll has a broad reef flat varying from less than 1,000 to more than 2,000 feet in width; the outer edge of this flat is marked by a shallow trough, then a higher coralline ridge which breaks up into a series of well-developed surge channels; beyond the end of the surge channels is a narrow sloping shelf from 10 to 40 feet deep which drops off abruptly into deep oceanic water. The lagoon is shallow with the deepest measured point about 50 feet, while most of the lagoon area is less than 12 feet deep; large areas near the islands were exposed by low tides. The western edge of the lagoon was marked by an interrupted series of coral reefs growing to near the surface with shallow passes between them. The western edge of the atoll drops off into deep water almost as abruptly as does the eastern except off the northern anchorage to the west of the chief village. (Dr. Cloud has an extensive report on the physical and biological condition of the atoll in his 1952 report.)

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COLLECTIONS AND HABITATS

Unfortunately the collections from Onotoa are not very extensive. An infected leg prevented me from collecting for about half of the 10 weeks we spent on the island, and in the short time remaining I had to devote full time to making the ecological survey, collecting snapping shrimps only as they appeared in the transects. Most of this small collection was lost in transit from Onotoa via Kwajelein to Hawaii and, eventually, only a small proportion of the shrimp collected at Onotoa arrived in Honolulu. Most of the specimens available for study were those collected by Dr. Cloud incidental to his studies on marine geology. However, small as the collection is, it is important for there have been no previous records from the Gilbert Islands.

On the windward reef a transect 20 feet wide was taken normal to the beach line and reaching to the coralline ridge, 750 feet to seaward. Because of the great diversity of habitats and the limitations of time no extensive transects were taken on the lagoon side, but the different shallow water habitats were sampled individually (see Banner and Randall, 1952).

The ecological notes on the stations from which shrimp were examined (it should be noted that alpheids were collected at almost all stations) are as follows:

A-1. Starting with division between beach rock and higher sand beach, at about 2.4-foot tide zone, reaching seaward 50 feet; substrate of consolidated reef limestone; many tide pools, the largest up to 20 feet long, 5 feet wide, and 6 inches deep; both exposed areas and bottoms of tide pools with scattered loose rocks up to a foot square and 6 inches thick.

A-2. 50–100 feet from beach line, about 2.2-foot tide zone; similar in nature to A-1, but with fewer and smaller tide pools.

A-3. 100–150 feet from beach line, about 2.0-foot tide zone; substrate similar to A-1, A-2.

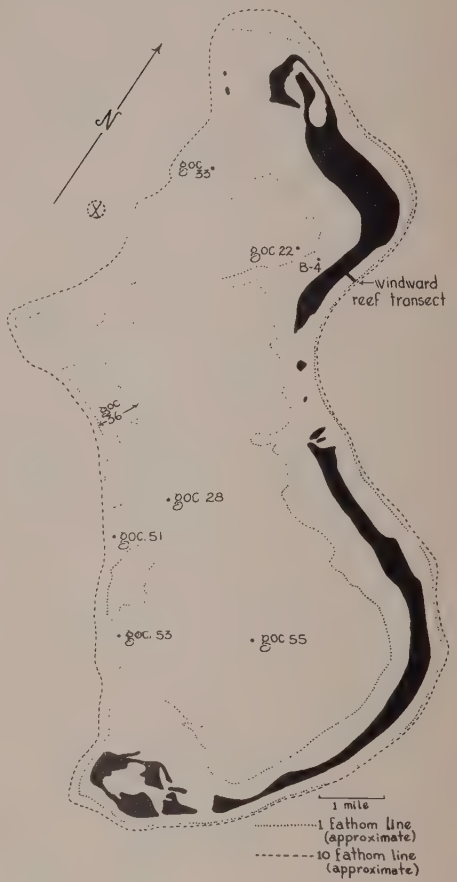

FIG. 1. Onotoa, Gilbert Islands, showing localities of collections. Place indicated by X is approximately 1°47′ S., 175°29′ E.

A-6. 250–300 feet from beach line, about 1.4-foot tide zone; about 30 per cent of area covered by very shallow tide pools. Substrate changing to a coralline algal sheet overgrowing dead coral heads *in situ* with numerous small holes passing through sheet into cavities remaining between the coral heads.

A-7. 300–350 feet from beach line, about 1.2-foot tide zone; about 80 per cent of the area covered by 1-inch-deep tide pools that only drain on extreme low waters; substrate as in A-6, but carpeted by thick tufts of green

algae in which many animals live; some loose rocks, several feet across, under which most of the snapping shrimp were collected.

A-10–12. 450–600 feet from beach line, 0.8–0.4-foot tide zone; about 70 per cent covered with shallow tide pools; substrate, algal covering, rocks similar to A-7; collecting of shrimp done at random in area wherever larger rocks occurred.

B-4. On sandy lagoon beach, about 0.0–2.0-foot tide level, 400 to 1,000 feet from high tide line; snapping shrimp collected from heads of dead coral protruding above the sand beach.

The following stations were those made by Dr. Cloud in which alpheids were collected:

GOC-22. Station nearly in line with B-4 above, 1,200 feet from shore, about 4–6 feet deep at low tide; substrate similar to B-4, but with more living and dead coral.

GOC-28. Middle lagoon region, from reef patches from 6 to 14 feet deep.

GOC-33. On outer edge of outer or western reef growth; depth from surface (low tide) to about 24 feet; flourishing coral growth.

GOC-36. Across surface of outer or western reefs; depth less than 6 feet; extensive coral growth separated by sandy areas.

GOC-51. In pass through western reef front; collections from coral patches, mostly of living coral, rising from sandy bottom at 15 feet to within 8–10 feet of surface.

GOC-53. A pass similar to GOC-51, bottom at 18 feet, coral patches rising to within 4 feet of surface.

GOC-55. From deepest spot on lagoon, sounded at 50 feet; bottom sandy to muddy, with scattered low clumps of living and dead coral.

SYNALPHEUS Bate

Synalpheus carinatus (de Man), 1888

For synonymy see Banner, 1957.

LOCALITY: 5 specimens from GOC-53.

DISCUSSION: These specimens, from the same collection that yielded the obviously closely related *S. consobrinus* de Man, have not been assigned to any of de Man's three subspecies. The subspecies were erected primarily upon the size of the ova, which were reported by de Man to be 0.4–0.5 mm. long in one subspecies, 0.9–1.0 mm. in the second, and 1.2 mm. long in the third. Of these specimens, three were ovigerous with the egg diameters 0.6 by 1.0 mm., 0.65 by 1.1 mm., and 0.65 by 1.5 mm. It was also noted that the smallest eggs had the youngest embryos, while the largest had embryos near hatching. The other characteristics used by de Man in the separation of the subpsecies are based on slight differences of variable characteristics, like the notch in the rostral carina, which in even these few specimens varied from well developed to almost nonexistent. Without other less variable characteristics to base the differentiation upon, with this group of specimens showing marked variation in the size of the ova, and with poecilogony well known among the synalpheids (see Coutière, 1899:444 *et seq.*) it appears that the division of the species into subspecies on these criteria is unrealistic.

Synalpheus amboinae (Zehntner)
Fig. 2

Alpheus amboinae Zehntner, Rev. Suisse de Zool. 2: 202, pl. 8, fig. 23 a, b, 1894.

Synalpheus amboinae de Man, Siboga Exped. $39a^1(2)$: 203, pl. 6, fig. 20, 1911.

LOCALITY: 3 specimens at GOC-53.

DISCUSSION: While these specimens are within the range of variation given by de Man (1911) and agree well with the descriptions of the species, they have been referred to this species with considerable doubts. They were collected at the same locality and at the same time as *S. consobrinus* de Man. These two nominal species differ in the presence of a tooth above the articulation of the large chela, and in slightly different proportions and armature of the telson. With the difference in the size of the specimens reported by de Man, one would suspect that

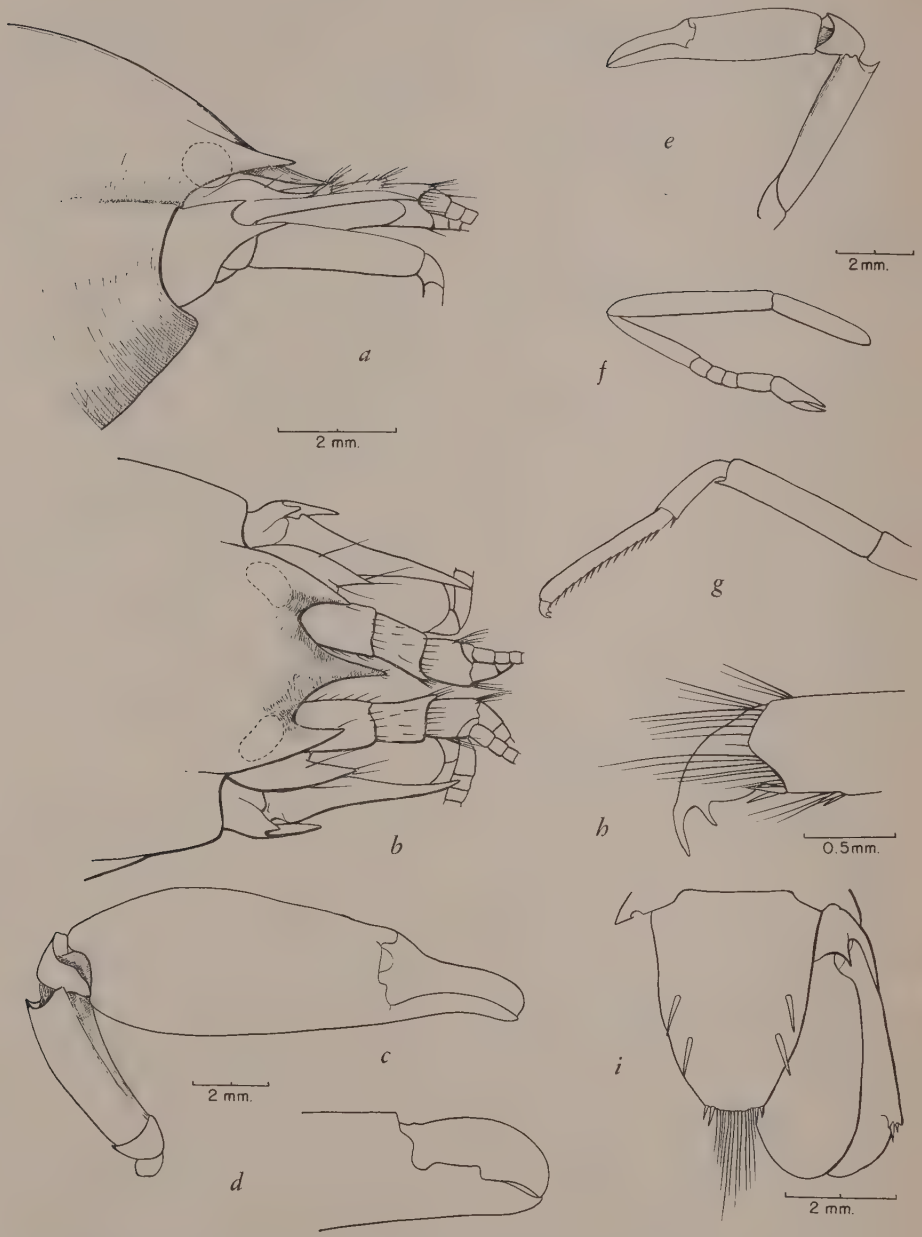

FIG. 2. *Synalpheus amboinae* (Zehntner). *a*, *b*, Anterior region; *c*, large cheliped; *d*, large chela, ventral view; *e*, small cheliped; *f*, second leg; *g*, third leg; *h*, third leg, dactylus; *i*, telson and uropods.

possibly the distinguishing characteristics may be the result of growth and therefore not of specific worth. However, in the absence of an intergrading series of specimens, the species has been permitted to stand.

Synalpheus consobrinus de Man, 1909

For synonymy see Banner, 1957.

LOCALITY: 8 specimens from GOC-53.

DISCUSSION: This group of specimens agreed well with the description and variations of de Man. In general they ran smaller than de Man's type, mostly 15 mm. long or less, yet the females were ovigerous. It is likely that these, together with the closely related *S. carinatus* (de Man) and *S. amboinae* (Zehntner), were collected from crinoids on the coral reef, but the exact data on their capture is lacking. In any case, it seems strange that three different but most closely related species would be living so close to each other in the same basic habitat, and for that reason alone the species should be suspect.

Synalpheus charon (Heller), 1861

Synalpheus charon obscurus Banner, 1956

For synonymy see Banner, 1956.

LOCALITY: 3 specimens at GOC-51, two of *S. charon charon*, one of *S. charon obscurus*.

DISCUSSION: The fact that in this locality two specimens of *S. charon charon*, an ovigerous female and a male, and one ovigerous female of *S. charon obscurus* were found casts some doubts as to the validity of the subspecies. It may indicate that the two forms are mere variations that breed together; it certainly indicates that the subspecies are not geographically distinct, as the other collections have indicated until now. However, as no intergrading specimens have been found and as the data of this collection do not indicate that the two subspecies might not maintain an ecological isolation, it has been decided to leave the subspecies standing.

Synalpheus paraneomeris Coutière, 1905

For synonymy see Banner, 1956.

LOCALITIES: 3 specimens at A-7; 5 specimens at GOC-51.

Synalpheus coutièrei Banner, 1953

For synonymy see Banner, 1957.

LOCALITY: 1 specimen at GOC-55.

Synalpheus pachymeris Coutière, 1905

Synalpheus biunguiculatus var. *pachymeris*, Coutière, Fauna and Geog. Maldive and Laccadive Archipelagoes 2(4): 873, pl. 71, fig. 9, 1905.

Synalpheus pachymeris de Man, Siboga Exped. 39a[1](2): 199, 1911.

LOCALITY: 1 ovigerous female from GOC-55.

DISCUSSION: This sole specimen agrees well with Coutière's description and figures except that the orbital teeth are more pointed, with their outer lateral margins concave, similar to that figured by de Man for *S. quadrispinosis* de Man (1911: pl. 12, fig. 57).

Synalpheus quadrispinosus de Man

Synalpheus quadrispinosus de Man, Nederland. Dierk. Ver., Tijdschr. II, 11(4): 298, 1910; also, Siboga Exped. 39a[1](2): 285, pl. 12, fig. 57, 1911.

Synalpheus quadridens de Man, Nederland. Dierk. Ver., Tijdschr. II, 11(4): 299, 1910; also, Siboga Exped. 39a[1](2): 284, pl. 12, fig. 56, 1911.

LOCALITY: A male and an ovigerous female from GOC-55.

DISCUSSION: de Man described as separate species two closely related forms, both characterized by four spines on the posterior margin of the sixth abdominal segment. They were separated by a series of characteristics, but principally by the form of the orbital teeth and the proportions of the small chela.

The first species, *S. quadrispinosus*, was represented in the Siboga collections by three mature and two immature specimens; the second, *S. quadridens*, by a sole ovigerous specimen lacking the large chela.

In this collection there is a single pair of specimens in good shape (although the male is lacking the small chela) which exhibits a perplexing confusion of the supposedly distinguishing characteristics of the two species as separated by de Man.

Rostrum and orbital hoods: Both specimens are very similar to *S. quadridens*, with the orbital teeth heavy and inflated.

Antennular peduncles: In both specimens the antennular peduncle is assymetrically developed, with the total lengths of the peduncles approximately the same, but with the basal article of one about 1.3 times the length of the other; however, in general appearance the articles are slender as reported for *S. quadrispinosus*. The stylocerite, to the contrary, is shorter and heavier like that of *S. quadridens*.

Antennae: In the male the scaphocerite is shorter than that of *S. quadrispinosus* (which had the shorter scaphocerite of the two species), while in the female it is longer than *S. quadridens*. The carpocerite is shorter in the male than *S. quadridens* (which had the shorter carpocerite), while in the female the article is long as in *S. quadrispinosus*.

Large chela: In *S. quadrispinosus*, the only species with the large chela described, the chela had a breadth of 0.34 its length, the fingers occupied the distal 0.27 of the length (recalculated from de Man), and there was a rounded-to-acute tooth above the dactylar articulation. In both of these specimens the proportions are exactly the same, with the breadth 0.25 the length, and with the fingers occupying the distal 0.26 the entire length of the chela. In the male the tooth above the the dactylar articulation is heavy and very conspicuous; in the female it is less pronounced and more gradually rounded.

Small chela: This is present only in the female, where the carpus equals 0.33 the length of the chela; the chela is 3 times as long as broad, with the fingers occupying the distal 0.4. These proportions are close to those of *S. quadrispinosus*, but not exactly the same.

Third leg: In the merus, where the greatest differentiation occurs, the male is 3 times as long as broad, like *S. quadridens*, and the female is 3.8 times as long as broad, like *S. quadrispinosus*.

Sixth abdominal segment: In the male the lateral teeth are narrow and acute like *S. quadrispinosus*, while in the female they are broader and more rounded like *S. quadridens*.

Telson: The proportions are given in the table below:

	MALE	FEMALE	*S. quadrispinosus*	*S. quadridens*
Length/breadth at base	1.3	1.3	1.5*	1.4*
Breadth at base / Breadth at tip	1.7	2.0	2.1–2.2	2.0
Length/breadth at tip	2.3	2.5	3.2–3.4	2.8

(*Ratios calculated from other ratios.)

From this it can be seen that the telson most closely approached that described for *S. quadridens*.

In summation, the specimens at hand are quite similar to *S. quadridens* in five characteristics, and to *S. quadrispinosus* in five other characteristics. Even if these were not a cohabiting pair—which they probably were, considering that they were collected together—their essential similarities would not permit them to be considered as other than a single species. Their similarities to both *S. quadrispinosus* and *S. quadridens* should establish their close relationship to both of de Man's species and their variable and inconsistent differences with both of de Man's nominal species destroys the validity of de Man's criteria for separation of the two forms. The only conclusion that may be drawn logically is that *S. quadridens* is a synonym (by page priority in the original description) for *S. quadrispinosus* and that *S. quadridens* is a variable species.

ALPHEUS Fabricius

Megacheles Group

Alpheus oahuensis Banner, 1953

Fig. 3

For synonymy see Banner, 1956.

LOCALITY: 1 specimen at GOC-51.

DISCUSSION: It is with considerable doubt that this specimen, a 11 mm. male, is assigned to this species. The rostral front, assymetrically developed in this specimen, is the same as that of *A. oahuensis*, but the articles of the antennular peduncles are shorter and relatively broader. The merus of the large chela is also slightly heavier, and the tooth on the distal inferior margin is rounded and without an acute tooth. The chela itself is of the same basic form, but more slender in this specimen, and the teeth flanking the articulation of the dactylus are not as well developed. In the small chela the greatest differences occur, for there the fingers are equal in length to the palm, instead of being 1.4 times as long, and are not thin and markedly curved, and do not bear the rounded ridge like those of *A. oahuensis*. The second legs are lacking. In the third legs the armature is similar but the legs are thinner, with merus 6 times as long as broad instead of 3.4 times; the inferior unguis of the dactylus is smaller.

But in spite of these differences in proportion, the general appearance of the two forms is quite similar, and these differences may be due to growth differences, for the type is 27 mm. long. Moreover, some variation along these lines was found in the paratypic series from Hawaii. However, with only one specimen from Onotoa, it is impossible to decide whether these differences are from individual variation, or from growth, or are constant differences that would be worth subspecific or specific rank.

Alpheus collumianus medius Banner, 1956

For synonymy see Banner, 1956.

LOCALITY: 2 specimens at GOC-51.

Machrochirus Group

Alpheus gracilis var. **simplex** (Banner), 1953

For synonymy see Banner, 1956.

LOCALITY: 1 specimen from GOC-51.

DISCUSSION: This single specimen agrees

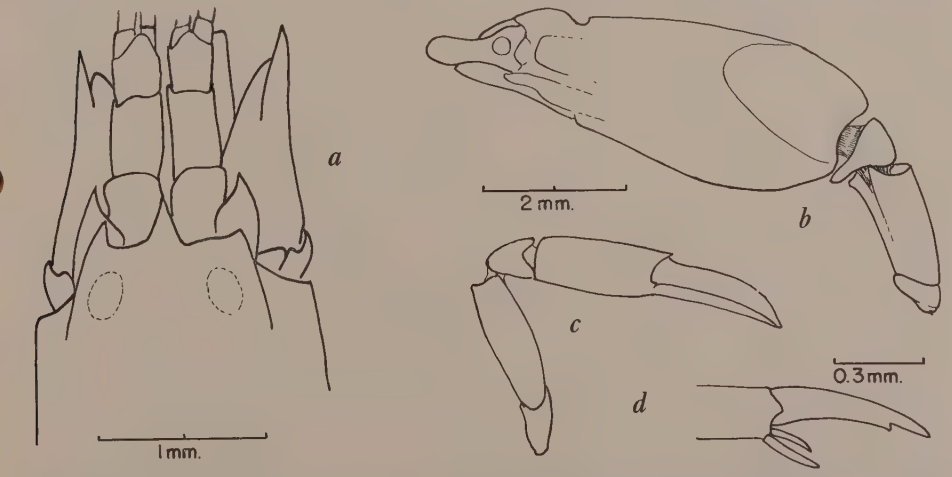

FIG. 3. *Alpheus oahuensis* (Banner). *a*, Anterior region; *b*, large cheliped; *c*, small cheliped; *d*, dactylus, third legs.

well with the specimens from Hawaii except that the dactylus of the third leg, instead of bearing a smooth convexity, carries an exceedingly small secondary unguis similar to but smaller than that found in *C. brachymerus* Banner (1953: fig. 17j). The external spine of the uropod is colorless.

In considering the specimens from Saipan, Arno, and Onotoa, the question arises as to the taxonomic level of this form. Obviously it is closely related to the *A. gracilis* of Heller, but whether it should be considered as a separate species, as a geographical subspecies, or as a variant—as it is now described—of the basic species, cannot easily be determined. On the basis of the Hawaiian specimens alone it would appear to be a separate species, or at least a subspecies; however, the specimens are from wide-spread localities. Yet none of these extra-Hawaiian forms are exactly like the specimens from Hawaii. It is possible that each of the studied archipelagoes have populations that could be interpreted as separate subspecies; certainly these variations appear to validate the consideration of these or the Hawaiian form as separate species. Yet the samples from the other archipelagoes have not been extensive enough to draw any conclusions about the populations, and many areas of the Pacific have not been sampled at all. Therefore the form is left as it was described originally with a tentative infrasubspecific designation.

Alpheus ventrosus Milne Edw., 1837
Fig. 4

For synonymy see Banner, 1956.

LOCALITIES: 2 specimens at GOC-22, 1 at GOC-33, 2 at GOC-36, 7 at GOC-51.

DISCUSSION: This group of four specimens from GOC-51, together with specimens from Saipan and Arno, present an almost complete intergradation between the small (8.5 mm.) form described as *A. latipes* and *A. ventrosus* which reaches a length of more than 40 mm.

While *A. latipes* as described was similar in general configuration to *A. ventrosus*, the two were distinctly separated by a number of characteristics. In the front of the carapace in *A. latipes*, the rostral carina was narrow and sharp and the orbital teeth were confluent with the front of the carapace, instead of having a flattened rostral carina and having the orbital teeth demarked medially from the margin of the carapace as is a characteristic for *A. ventrosus*. The antennular peduncles and the second and third legs were relatively broader in *A. latipes*. The fingers of the large chela were relatively shorter and the merus of the large and small chela were feebly armed or completely unarmed in *A. latipes*, instead of bearing up to four or five strong spines as in *A. ventrosus*. The dactylus of the third leg was abruptly curved and strongly acute in *A. latipes*, while in *A. ventrosus* the dactylus was of a peculiar blunt form, with a growth on the tip that was reminiscent of a horse's hoof. (Compare figs. 27, 28, Banner, 1953.)

Yet this group of specimens, ranging in size from 8.2 mm. to the adult size, shows an almost perfect intergradation in all of these characteristics. Two of the most marked and most easily observed characteristics, the form of the rostral carina and the form of the dactylus of the third legs, were chosen for detailed study and illustration. The four smaller specimens came from the station mentioned above; the last specimen came from the author's collection from Saipan.

In the 8.2 mm. specimen, the parts are almost identical to those described for *A. latipes;* if anything the dactylus of the third leg is slightly thinner. In the 9.7 mm. specimen the rostral carina does not change, but the dactylus is developing some extra superior and inferior thickenings. In the 10.8 mm. specimen the rostral carina still remains constant, but the dactylus is definitely growing thicker, the hook smaller, and a superior and inferior ridge is becoming more marked. In the 15.4 mm. specimen the interorbital portion of the rostral carina is broader and flatter,

but it still remains a sharp ridge on the anterior portion of the rostrum; the dactylus retains only a trace of the hook, the superior ridge is well developed, and the "hoof" is developing; the last spines of the propodus are thickening, but remain acute. Finally, in the 16.2 mm. specimen, the rostral carina is flattened to the tip of the rostrum, but still not as broad as in the full-sized adults; the dactylus has the structure and proportions of the adult; the last spines of the propodus are broad, distally rounded, but are not yet excavate on the tips, as are found in the mature specimens.

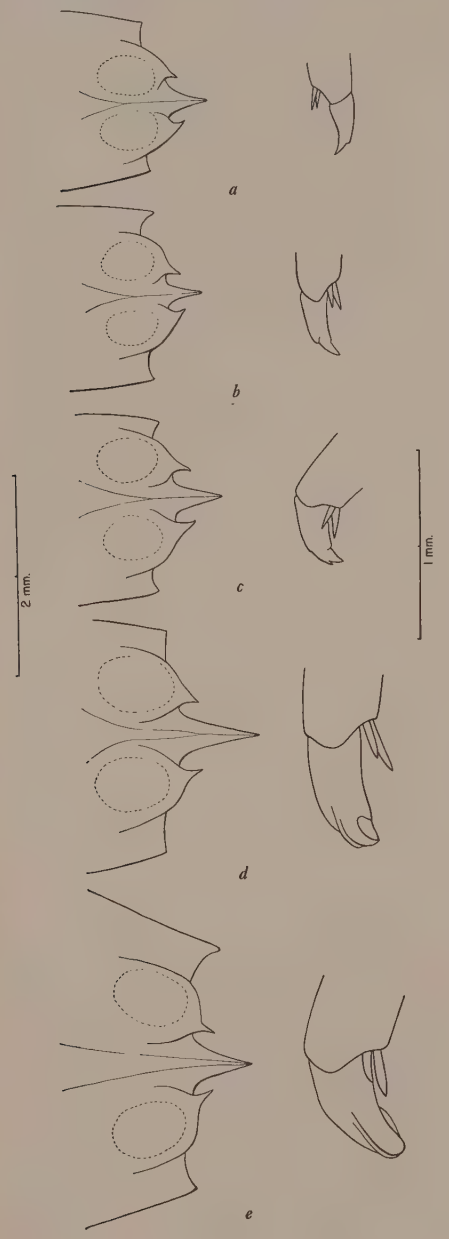

FIG. 4. *Alpheus ventrosus* Milne-Edwards, to show developmental stages in the form of the rostrum and dactylus of third legs. *a*, An 8 mm. specimen; *b*, a 9.7 mm. specimen; *c*, a 10.8 mm. specimen; *d*, a 15.4 mm. specimen; *e*, a 16.2 mm. specimen; *a–d*, from Onotoa, *e*, from Saipan; carapaces and dactyli with separate scales. (Note: Dactyli are in differing rotation.)

This easily recognizable species, possibly one of the most distinctive species of the genus, appears to have had at least four names applied to it: *A. lottini* Guérin, 1826–1830 (by Stebbing and Barnard, see below); *A. ventrosus* H. Milne-Edwards, 1837; *A. laevis* Randall, 1839; and finally, as discussed above, *A. latipes* (Banner), 1953. Unfortunately the three early names were published with short and generalized descriptions; only the first description carried a figure, and this figure appears to have been inaccurate.

The type specimen of Randall for *A. laevis* probably is lost with the rest of the early types at the Philadelphia Academy of Science, but there is only one species from Hawaii, the type locality, that meets the qualifications of his description including that of size ("length about 1½ inches"). This species is the one now known as *A. ventrosus* (see Banner, 1953: 84, fig. 28).

The type of Milne-Edwards has never been formally redescribed, but its characteristics are known. In *Les Alpheidae* (1899) Coutière discusses and figures specimens that he identifies as *A. laevis* Randall (figs. 54, 201, 251, 263, 307, 324, 325), a form plainly identical with the species from the Hawaiian Islands that has been known as either *A. laevis* or *A.*

ventrosus. Especially noteworthy are the figures of the dactyli of the third to fifth legs (fig. 324, 325), a characteristic unique within the genus. Then Coutière (1905: 882) reported, "L'examen des types de H. M. -Edwards ne permet aucun doute sur l'identite de l'*A. ventrosus* et de l'espece . . . *A. laevis* . . . Randall." There appears, therefore, no question but that the two names refer to the same species.

However, there is considerable question whether the form described as *A. lottini* by Guérin is this species. Milne-Edwards in his original description of *A. ventrosus* (1837: 353) concludes by saying, "L'Alphée de Lottin dont il a été publié une bonne figure, mais dont la description n'a pas encore paru [the named figure was published about 10 years before the printed description, and in the meantime Milne-Edwards published his work] paraît être très-voisine de l'espèce précédete." Stebbing (1915: 82) states, "But the description of *A. ventrosus* does not seem to justify any claim for the priority of that name over Guérin's *A. lottini*." He therefore used the name *A. lottini*. Barnard, in his work on the South African decapods (1950: 748, fig. 141), follows the name used by Stebbing.

However, while Guérin's description and figures do not show any great differences from the generalized description of Milne-Edwards, they do show differences from the form now recognized as *A. ventrosus* and from the types as re-examined by Coutière. In Guérin's figure, the rostrum in *A. lottini* reaches almost to the middle of the second antennular article, instead of to the distal part of the first article; the lateral spine of the scaphocerite is too prominent; the second article of the carpus of the second leg is about 6 times as long as broad instead of 1.5 times; the dactyli of the third to fifth legs are shown as definitely acute. In the description, the wrist of the large chela is described as elongate and cylindrical, instead of short and cyathiform, and the articles of the posterior legs also as cylindrical instead of compressed. A most important difference is in the color, for the orange-red color of the specimens of this species is one of the most constant colors found in the family, yet Guérin figured it as a delicate lettuce green and described it, "Sa couleur est d'un jaune verdâtre dans l'alkool." It should be noted, too, that the figure does not show any orbital spines, yet Guérin reports these in his description.

It is possible, of course, that these differences are the result of inaccurate description and the types are actually identical; however, there is no reason to presume so, and until the time that Guérin's types are found and redescribed, the name *A. ventrosus* should be accepted. If the types of Guérin should be found to be identical with the species of Milne-Edwards, I would recommend that the little-known and almost never used earlier name be suppressed by the International Commission on Zoological Nomencalture in favor of the long accepted name of this, one of the most common species of the genus.

One other problem raised by Stebbing (*loc. cit.*) was concerned with the identity of the form that Bate called *A. laevis* Randall. "Bate's figure of *A. laevis* in the 'Challenger' report [1888: 555, pl. 99, fig. 3] cannot easily be reconciled with the species here in question." It is true that Bate neither figured nor described the peculiar unguis of the third to fifth legs, and he depicted the legs, especially the second legs, as much too thin, but otherwise the specimen appears to agree with *A. ventrosus*. Moreover, Bate's specimen came from Hawaii, and, as with Randall's specimen, there are no other species in Hawaii that agree even moderately well with the description and figures. It should be noted, also, that Bate shows dark spots on the superior surface of the large and small chelae, a characteristic of the species (cf. Banner, 1953: fig. 28 c, d, e).

The last question that might arise is whether the species identified by Stebbing and Barnard from South Africa as *A. lottini* is the same as *A. ventrosus*. Stebbing's brief description leaves little room for doubt, and Barnard's

excellent figures make it certain that the South African form does not differ in any significant fashion from the Pacific form.

Alpheus machrochirus Richters, 1880

For synonymy see Banner, 1957.

LOCALITY: 1 specimen at GOC-36.

Obesomanus Group

Alpheus lutini Coutière, 1905

For synonymy see Banner, 1956.

LOCALITIES: 1 specimen each at B-4, GOC-28, GOC-53, GOC-55.

Alpheus species

LOCALITY: 2 specimens from GOC-51.

DISCUSSION: These two specimens, both females, appear to be in either the *Obesomanus* group or the *Crinitus* group, and may be related to the complex containing the species *A. obesomanus* Dana, *A. microstylus* (Bate), and *A. lutini* Coutière. These specimens appear to be of a separate species, possibly new, but preliminary work on the variation in this complex indicates that the specific characteristics need re-evaluation. Therefore it has been considered wiser to defer judgement on these specimens until a later paper when the related species are considered.

Crinitus Group

Alpheus brevipes Stimpson

Alpheus brevipes Stimpson, Acad. Nat. Sci. Phila., Proc. 12: 30, 1860.
Crangon brevipes Banner, Pacific Sci. 7(1): 103, figs. 35, 36, 37, 1953 [neotype described].

LOCALITY: 2 specimens at GOC-51.

Alpheus bucephalus Coutière

For synonymy see Banner, 1956.

LOCALITY: 3 specimens at A-7.

DISCUSSION: These specimens exhibited a variation similar to those forms reported upon from Saipan.

Alpheus clypeatus Coutière

Alpheus clypeatus Coutière, Fauna and Geog. Maldive and Laccadive Archipelagoes 2: 897–898, pls. 81, 82, figs. 36, 36g, 1905.
Crangon clypeata Banner, Pacific Sci. 7(1): 107, figs. 37, 38, 39, 1953.

LOCALITY: 1 specimen at GOC-51.

Alpheus pachychirus Stimpson, 1860

For synonymy see Banner, 1956.

LOCALITY: 1 specimen at GOC-51.

Diadema Group

Alpheus diadema Dana, 1852

For synonymy see Banner, 1956.

LOCALITY: 1 specimen at GOC-36.

Alpheus lanceloti Coutière

Alpheus lanceloti Coutière, Fauna and Geog. Maldive and Laccadive Archipelagoes 2: 900, pl. 83, fig. 39, 1905.

LOCALITY: 1 specimen only from A-3.

DISCUSSION: This specimen, fortunately intact, agrees almost perfectly with the original description by Coutière. The only exception may be in the merus of the large cheliped, which Coutière shows as bearing four immovable denticles proximal to the terminal denticle, and which bears four short but heavy movable spines in this specimen. It should also be noted that the merus of the third leg on one side is like that of the type bearing three movable spines, while on the other side there are four such spines.

Alpheus gracilipes Stimpson, 1860

For synonymy see Banner, 1956.

LOCALITY: 2 specimens from GOC-22.

Alpheus paracrinitus (Miers) **bengalensis** Coutière, 1905

For synonymy see Banner, 1956.

LOCALITY: 1 specimen from A-10–12.

Edwardsii Group

Alpheus crassimanus (Heller), 1865

For synonymy see Banner, 1957.

LOCALITY: 1 specimen from A-1.

DISCUSSION: This single specimen is a female and the identification therefore lacks the confirmation obtainable only by the examination of the small chela of the male; however, on all characteristics it is similar to specimens of the same size from Hawaii (see Banner, 1953: 134).

Alpheus strenuus Dana, 1852

For synonymy see Banner, 1957.

LOCALITY: 2 specimens from A-3.

DISCUSSION: Special efforts were made to collect large specimens, presumably of this species, from the windward reef flat where they were abundant. However, it was found impossible, for they lived in burrows in consolidated coral where a geologist's pick would scarcely penetrate.

The Gilbertese on the island informed me that they used this shrimp—and some other species of small chelate shrimp-like crustaceans that I was unable to collect—as food. The shrimp were caught in the back-ridge trough of the windward reef with nets at night when torch fishing. The collective name for this group of decapods is "Teniwarowaro," and my informant stated that their size ranged to 14 inches long (235 cm.). The Gilbertese ate the cephalothorax and abdomen of the shrimp.

Alpheus pacificus Dana, 1852

For synonymy see Banner, 1956.

LOCALITIES: 1 specimen at A-2, 40 at A-3, 3 at A-6, 1 at A-7.

DISCUSSION: Some of the specimens placed in this species are lacking their small chela, so that they can not be positively identified. However, they agree on other characteristics with the complete specimens.

In this group of specimens slight sexual dimorphism is found in the proportions of the small chela, with the fingers of the male being relatively longer (up to twice as long as the palm) than in the female where they are about 1.5 times as long as the palm. However, the characteristic is not constant, especially for those of smaller size where the finger length is quite variable between these limits.

Alpheus dolerus Banner, 1956

For synonymy see Banner, 1956.

LOCALITY: 2 specimens at GOC-51.

DISCUSSION: The finger of the small chela of the male bears a fringe of setae that reaches about half the length of the dactylus on the inner face and a shorter distance on the outer face; this fringe is similar to the fringe of the "balaeniceps-type" chela except that it is continuous over the superior surface of the finger and the finger is not expanded. Otherwise the two specimens are like those of Saipan.

Alpheus parvirostris Dana, 1852

For synonymy see Banner, 1956.

LOCALITIES: 1 specimen at GOC-22, 3 at GOC-28.

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[3] These are the title page dates, and evidently do not correspond to the actual dates of publication; however, there appears to be some difference of opinion as to the actual dates of publication.

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THE BLOOD VOLUMES OF SOME REPRESENTATIVE MOLLUSCS

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THE BLOOD VOLUMES OF SOME REPRESENTATIVE MOLLUSCS*

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The reports of measurements of the blood volume of molluscs found in the literature are few in number and represent, in most cases, incidental observations rather than results of specific investigations. Leitch (1916) obtained by the bleeding of *Planorbis* a volume of blood which was 33% of the tissue weight. In 1931, Borden determined the blood volume of *Planorbis corneus* using the Welcker method and obtained a value of 58% of the total tissue weight. This higher value is to be expected, since it is impossible to remove all the blood from an animal by simple bleeding. In 1950, Martin & Huston, using the inulin dilution method, reported the blood volume of *Aplysia californicus* to be 83·3% of the total weight. This is not surprising since *Aplysia* has a very extensive haemocoele, and more than half the body weight in blood can be drained in a few minutes from a single incision into the animal.

Prosser & Weinstein (1950) determined the blood volume of two fresh-water molluscs, *Lampsilis ventricosa* and *Amblema costata*, and found a very low value. In terms of percentage of total wet tissue weight, their average values for experiments done with sodium thiocyanate and with T-1824 are very close to 8%. These results are not verified by the brief report of Potts (1954) who, in studying the rate of urine production in *Anodonta cygnea*, found a mean inulin space of four animals to be 55% of the wet tissue weight.

Since the values which have been reported are so few in number and are so wide in range a more extensive study of the subject appeared to be of value. The purpose of this investigation was to measure the total volume of circulating fluid in members of various groups of molluscs. From the results obtained the extent of variability within certain species and, to some extent, within the phylum can be ascertained.

GENERAL METHODS

Blood volumes were determined by measuring the dilution of a known quantity of material injected into the circulating fluid. In order to facilitate rapid mixing of the

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material, it is desirable to make injections directly into the heart or a major blood vessel. When it was impossible to use this technique, as was the case in about half the forms investigated, the injections were made directly into the haemocoele.

Table 1. *Summary of experimental procedures*

Animal	Wet wt. without shell (g.)	Vol. of initial normal blood sample (ml.)	Material injected	Vol. injected (ml.)	Vol. of each serial sample (ml.)	Sampling interval (min.)	Total length of experiment (min.)
Cryptochiton stelleri	421–877	5–7	Inulin, 0·4 % in blood	5	0·5	30–60	300
Aplysia californicus	146–411	2–5	Rabbit Hb 3 % in blood	7–20	2	15–30	60–235
			Inulin, 0·5 % in blood	6–10	1–2	15–30	180–400
			0·05–0·10 % silver proteinate in blood	8–15	1–2	15–30	85–400
Archidoris sp.	50–62	0·5–1·0	Inulin, 0·5 % in sea water	1–2	0·2–0·5	20–30	240–360
Achatina fulica	41–74	1·0	Inulin, 0·5–1 % in distilled water	1	0·2	15–20	130–270
Arion ater	10·0–43·7	0·15	Inulin, 4 % in distilled water	0·20	0·05	10–60	120–480
Mytilus californianus	39–122	1–1·5	Inulin, 1 % in sea water	0·5–1·0	0·2	15–30	180–250
Margaritana margaritifera	31–49	0·5–1·0	Inulin, 1 % in distilled water	0·5–1·0	0·15–0·20	15	120–180
			T-1824 bound to rabbit serum	0·5–1·0	0·15–0·20	15	120–180
Octopus hongkongensis	4550–22500	7–10	T-1824, 0·1–0·375 in sea water	3–15	1·0–2	15–30	135–540
			HgS, 2·3 % in sea water	10	1·0	30	450–495
			Inulin, 4–5 % in sea water	8–100	1·0–2	15–30	360–540

A number of agents were tested as to their applicability in blood volume determinations. These included various dyes, haemoglobin, haemocyanin, mild silver proteinate, sucrose, and inulin. The most satisfactory material used was inulin as it did not appear to complex with any portion of the circulating fluid and was only slowly excreted. The material to be injected was prepared by diluting a stock aqueous solution with blood from animals of the same species or with a fluid of approximately the same osmotic concentration as the circulating fluid. At the beginning of the experiment enough normal blood was withdrawn for preparation of blanks and standards. The volume of test solution injected approximately replaced the amount of blood removed for this purpose. Samples of blood were taken at appropriate intervals over a period of time which preliminary experiments had shown to be sufficient for uniform distribution. Before taking each sample a quantity of blood larger than the dead space of the sampling tube was removed and subsequently re-injected. In Table 1 are summarized the specific details concerning the experimental procedure followed for each form investigated.

Standard analytical procedures were used in the determination of the concentra-

tion of the materials employed. For those analyses which required a protein-free filtrate, the proteins were precipitated by the Somogyi method (1930). The concentration of various dyes and other coloured materials were determined by comparison of the optical density of the experimental samples to those of standard solutions of known concentration which were prepared simultaneously. Inulin was analysed by the Harrison method (1942) and the anthrone method (Morris, 1948). For the determination of inulin in samples from animals with a relatively high blood sugar, a yeast digestion was employed with the Harrison method and a NaOH digestion (Little, 1949) with the anthrone method to remove the interfering sugar. The instruments used in different laboratories included the Beckman DU spectrophotometer, the Klett–Summerson photoelectric colorimeter and the Cenco Photelometer.

For the calculation of blood volume the general procedure was to plot the optical density of the samples against time. Since the concentration of the injected material decreases exponentially with time, semi-logarithmic graph paper was used to obtain a linear relationship. The line obtained was extrapolated back to zero and the concentration in grams per millilitre equivalent to the optical density at zero time was determined from a standard curve. The volume of fluid into which the injection had been made originally was obtained by dividing the weight of the injected material by the concentration at zero time. This volume of fluid was then expressed as percentage of the wet weight of the animal without shell.

Blood volumes, cell water volumes and total water volumes are expressed as 'mean $\pm$ standard deviation %' of the wet weight of the animal or tissue.

ANIMALS AND SPECIAL METHODS

A. *Placophora*

The volume of circulating fluid was determined in the large chiton, *Cryptochiton stelleri*. The animals which occur in abundance along the shores of Puget Sound were collected at approximately 3-month intervals. They were maintained at 8–10° C. in 150 gal. aquaria through which filtered sea water was recirculated at a rate of approximately 1 l. per minute per aquarium. A description of the sea-water system in which these animals were kept may be found in Thompson (1935). Under these conditions the animals survived in healthy condition for as long as 2 years.

In preparation for an experiment each animal was removed from the sea water, blotted dry and weighed. In this form, blood samples were taken through a plastic catheter placed in the haemocoele. For insertion of the catheter, a needle of smaller diameter was forced through the body wall in a region below the middle shell plates. The plastic catheter stiffened with an internal wire was then pushed through the opening, the internal wire was withdrawn and the tube plugged to prevent bleeding. This technique resulted in a leak-proof opening into the haemocoele through which fluid could be injected or withdrawn. Blood samples were often expressed from the catheter under pressure, not from action of the heart but from contraction of the body wall. Occasionally blood contaminated with eggs was obtained; such an animal was omitted from the report. Many specimens were dried to constant weight at 105° C. and the total body water calculated after correcting for the shell weight.

B. *Gastropoda*

(1) *Opisthobranchia–Tectibranchiata*

The sea hare, *Aplysia californicus*, was collected at low tide at about 2-week intervals on the Southern California coast in the vicinity of Corona del Mar. They were maintained on a diet of algae and kept in a running sea-water aquarium until needed.

At the beginning of the experiments the animals were wiped dry, weighed, and then a multiply perforated plastic catheter was inserted through an opening made in the body wall. The tube was tied in place with a ligature which caught up a small portion of the tissue. Since this animal is able to contract the body so as to close off a surprising length of tubing it was not until catheters of half body length were inserted that successful sampling became routine. Since there was a loss in weight due to the release of fluid from glands in the handling procedure and from the escape of blood during cannulation of the animal, another weight determination was made before the injection of the experimental solution. The weight determined at this time, corrected for the contents of the gut, was used subsequently in calculation of the blood volume. The injections were made into the ctenidial vessel, and samples were taken from the catheter in the body wall.

At the end of the experimental period the body wall was cut and the volume of blood which could be drained from the animal was determined. The contents of the digestive tract were removed and weighed and the animals dried to constant weight at 105° C.

(2) *Opisthobranchia–Nudibranchiata*

The sea lemon, *Archidoris* sp., was the animal used as a representative of the nudibranch molluscs. Specimens were collected intertidally along the shores of San Juan Island and were maintained in the all-glass sea-water system of the Friday Harbor laboratories.

The technique used for cannulation was the same as that described for *Aplysia*. However, in this form injections were made and samples subsequently withdrawn from the catheter in the haemocoele. Consequently, before the experimental samples were taken, the blood of the catheter was thoroughly mixed with the blood of the haemocoele by flushing it in and out several times. The amazingly effective reflexes of the animal sometimes resulted in contractions of the body wall which succeeded in shutting off all the apertures in the cannula. However, the animal would relax eventually and samples could then be taken successfully. At the end of the experiment the animals were bled and dried to constant weight in an oven at 105° C.

(3) *Pulmonata*

The giant African snail, *Achatina fulica*, was collected in the vicinity of Kaneohe Bay on the island of Oahu, Territory of Hawaii. The animals were kept in glass terraria provided with a sod bottom and given water and fruit *ad lib.*

The animals used in these determinations were anaesthetized with ether vapour in a closed container, the process taking about ½ hr. When the animals were sufficiently relaxed two stitches with linen thread were taken close together through the body wall slightly posterior to the genital aperture. Between the stitches a small opening was made and the soft plastic cannula inserted through the opening into the haemocoele. The cannula was tied securely in place to prevent leakage of blood. In about 30 min. the animals appeared to have recovered from the anaesthesia and began to crawl about in the customary fashion. At this time the injection was made into the haemocoele, and then at subsequent intervals of time blood samples were removed from the same cannula. At the end of an experiment

the shell, and any eggs that might be present were removed, the free blood was drained off, and the remainder of the tissue dried to constant weight.

The slug, *Arion ater*, was collected for the most part on the campus of the University of Washington, in Seattle, and at Friday Harbor. The individuals were maintained in terraria with an ample supply of water, lettuce and oatmeal.

Collection of blood and injection of materials were made through a plastic catheter which was inserted through the lateral body wall in the middle region of the animal. In this form it was not necessary to tie the catheter in place. If the animals were cooled to about 8° C. before the insertion of the catheter, the production of mucus and general body movements were decreased, making the operation easier.

In the early experiments, the dry weights of the animals were not determined. However, they were obtained for later experiments by the drying of the specimens to constant weight in an oven at 105° C.

C. *Pelecypoda*

Filibranchiata

The mussel, *Mytilus californianus*, was collected intertidally along the breakwater south of the entrance to Gray's Harbor, Washington. Specimens were kept in the Seattle sea-water system described earlier.

Preparation for the experiments was made without anaesthesia. After scraping away part of the hinge ligament, always working between known positions of the muscles, it was possible to break away small pieces of shell with a surgical rongeur or bone forceps. The larger part of the heart and most of the aorta were exposed by this method. It was necessary to remove the shell carefully to prevent damage to the body wall or muscles. The animal was weighed and then placed in a container of sea water of such depth as to leave only the exposed area free for injection and sampling. Operated animals which were not used for experimentation survived well in the laboratory and frequently would begin to deposit shell.

After such preparation a sharp 1 in. hypodermic needle of 26 or 27 gauge was introduced directly into the anterior aorta, and anchored in place with a piece of modelling clay resting on the shell of the animal. The test solution, coloured with T-1824, was injected into the aorta and the preparation carefully examined for leaks. During the course of the experiment, blood samples were withdrawn at appropriate intervals of time. At the completion of the experiment the animal was weighed and the volume of blood drained from multiple cuts in the region of the heart determined. The animal was removed from the shell and the weight of the shell recorded as well as the wet weight of the tissue. The dry weight of the tissue was determined after drying at 105° C.

Specimens of *Margaritana margaritifera* were obtained from the shallow waters of the Sammamish river in King County, Washington, about halfway between the origin of the river in Lake Sammamish and its termination in Lake Washington. They were kept in about 4 in. of water in a cement aquarium table through which Seattle tap water circulated at a slow rate. This water is chlorinated from time to time and does not contain much plankton. Experimental animals were not kept longer than a month before use, though some left in this aquarium survived for several months.

In preparation for an experiment a triangular section of a valve in the area of the heart and between the attachments of the adductor muscles was prised out after circumscribing the area with hacksaw cuts. The opening was extended if necessary by chipping away additional pieces of the shell. The animal was weighed and returned to a container through which fresh tap water circulated.

Since it is difficult to see the aorta in this form, a sharp 26 or 27 gauge hypodermic needle

was thrust through the pericardial sac into the lumen of the ventricle and supported firmly by a clamp. Into the end of the needle was inserted an adapter of small internal diameter to which was connected a 3 in. length of plastic tubing (1 mm. I.D.). Injections were made and samples were taken from the end of the plastic tubing. The use of this sampling device was advantageous, since it had a very small dead space and eliminated any movements during sampling which might destroy the fragile preparation. At the close of the sampling period weight and volume determinations were made similar to those for *Mytilus*.

D. *Cephalopoda*

Specimens of the large octopus in Puget Sound, probably *Octopus hongkongensis* Hoyle (Pickford, personal communication), were taken from their dens at low tide or captured on the rocks in shallow water. They were kept in large aquaria either at the Friday Harbor Laboratories of the University of Washington, or in the sea-water system at Seattle which has already been described. In most instances the animals ate the crabs or fish offered to them and could be kept indefinitely. In a few cases the animals would not accept food during the week or two which preceded their use in the experimental work.

Surgical provision was made for obtaining blood samples, usually the evening before the determination. The octopus was anaesthetized in about 30 gal. of aerated sea water by the gradual addition of 95% ethyl alcohol to a final concentration of about 2·5%. In 15–30 min. relaxation of the animal was obtained, at which time one of the branchial hearts could be drawn out of the mantle cavity without damage to the animal. An opening was made through the wall of the branchial heart and a soft rubber catheter passed through the lumen of the heart and into the lateral vena cava. It was secured with a ligature which enclosed a complete ring of tissue. The external end of the tube was closed with a solid glass stopper, the animal weighed and returned to normal sea water. In a short time the normal respiratory movements were regained, as well as muscular control. To prevent the animal from disturbing the tube, it became the practice to tie the tentacles in a net bag immediately after recovery from anaesthesia.

The next day, in preparation for an experiment, a small bore plastic tube long enough to hang over the edge of the aquarium was connected to the rubber catheter and the net bag was removed from the animal. Solutions were injected and blood samples were taken through the same tube which was adequately rinsed both for complete injection and to insure the taking of representative blood samples.

RESULTS

A. *Placophora*

Early experiments on *Cryptochiton* showed that a linear relationship between time and the logarithm of the concentration of the injected material was not obtained until 1–2 hr. had elapsed. The period of time necessary for this evidence of uniform distribution will be referred to subsequently as the equilibration time. In order not to remove too much of the injected materials before mixing had occurred, the first sample was taken after approximately an hour had elapsed. In Fig. 1 are given data from a typical experiment on the species. The long periods required for distribution of the injected material probably indicate a sluggish circulation in this animal. Although samples were taken for a long period, the possibility exists that not all the injection fluid entered regions through which free circulation occurred. It is thought that this might account for some of the variability in the results obtained. Since the

weight of the valves which make up the shell, and the total body water, were not determined for each experimental animal, these quantities were determined for a series of five normal animals. The valves averaged 5·2% of the total wet weight, and the total body water averaged 85·1 ± 2·2% of the wet tissue weight.

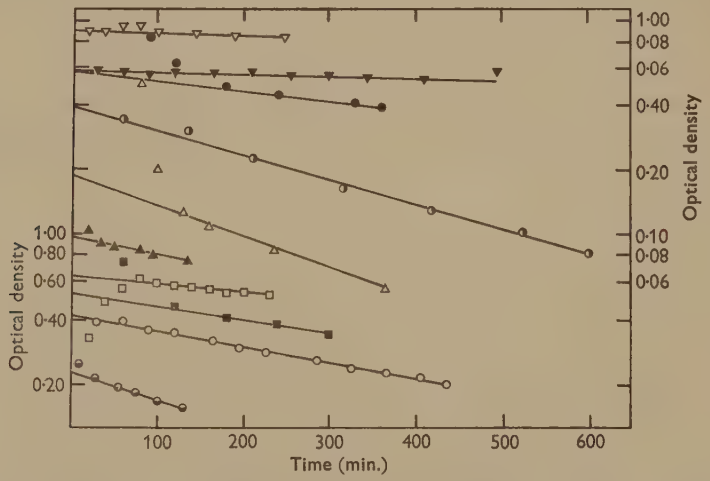

Fig. 1. Typical dilution curves following the injection of inulin into the circulatory system of the following animals: Group A. Use scale on right ordinate. Group B. Use scale on left ordinate. A: ▽, *Aplysia californicus;* ▼, *Octopus hongkongensis* (HgS); ●, *Octopus hongkongensis* (inulin); ◑, *Arion ater;* △, *Archidoris* sp. B: ▲, *Achatina fulica;* □, *Mytilus californianus;* ■, *Cryptochiton stelleri;* ○, *Octopus hongkongensis* (T-1824); ◒, *Margaritana margaritifera.*

In Table 2 the blood volumes of fifteen animals are given. The average blood volume was 43·8 ± 8·6%. Also included in this table are results of blood-protein determinations and values for cellular water. The protein was analysed by a micro-Kjeldahl method (Ballentine & Gregg, 1947). The percentage cellular water was calculated in the following manner. Since there is an open circulatory system in *Cryptochiton*, the volume of cellular water was obtained by subtracting the blood water from the total volume of water in the animal. To the volume of cellular water was added the dry weight of the animal, corrected for the shell and the salt and protein content of the blood, if this dry weight was significantly large. This sum theoretically should be equivalent to the wet weight of the cells in the tissues. The volume of cellular water was then expressed as percentage of the wet weight of the cells. The cellular water for this animal was 76·6 ± 3·4%. A discussion of the cellular water of this and the other forms studied will be deferred until later in the paper.

Table 2. *Inulin volume, blood protein and cellular water of* Cryptochiton stelleri

Wet wt. without shell (g.)	Blood vol. (ml.)	Blood vol. as % wet wt. without shell	Blood* water (ml.)	Blood protein g./100 ml.	Dry wt. without shell (g.)	Corrected† dry wt. (g.)	Cellular water as % wet tissue wt.
421	250	59·4	246	0·97	62·6	52·7	68·1
475	230	48·5	224	2·47	70·8	58·2	75·5
484	266	54·9	259	—	72·1	58·8	72·2
495	204	41·2	199	—	73·8	63·6	77·8
578	185	32·0	180	2·55	86·2	75·9	80·4
649	292	45·0	283	2·94	96·6	79·3	77·3
666	367	55·1	354	3·58	99·3	75·2	74·0
670	222	33·1	217	—	99·7	88·6	79·9
712	308	43·2	300	—	106·0	90·6	77·1
726	381	52·5	372	—	108·2	89·2	73·3
753	273	36·3	266	2·25	112·2	97·8	79·3
753	254	33·7	247	2·19	113·7	100·5	79·6
763	352	46·2	346	0·92	113·8	100·0	75·1
871	333	38·3	325	—	129·8	113·2	78·7
877	329	37·5	318	3·03	129·2	109·4	79·7
Mean	—	43·8	—	—	—	—	76·6
S.D.	—	8·6	—	—	—	—	3·4

* Blood volume corrected for the salt and protein content of the blood. The volume occupied by 3 g. salt was assumed be 1 ml. The partial specific volume of the protein was assumed to be 0·74 ml./g.
† Corrected for the salt and protein content of the blood. In those cases where the blood protein was not determined, was assumed to be 2·0 g./100 ml. The salt concentration was assumed to be 3·0 g./100 ml.

B. *Gastropoda*

(1) *Opisthobranchia*

(*a*) Aplysia californicus. The tectibranch, *A. californicus*, looks and feels like a bag of water. In specimens of a group of twenty-four animals, the mean fluid volume which could be drained off, expressed as percentage of body weight, was 60%, varying from 51 to 71%. It is, therefore, not surprising that analysis of the blood volume by dilution techniques yielded values higher than those of the other molluscs investigated.

A number of different agents were employed for the blood-volume determinations in *Aplysia* and the results obtained are presented in Table 3. Inulin gave a mean blood volume of $79{\cdot}3 \pm 3{\cdot}1\%$; haemoglobin gave $76{\cdot}2 \pm 4{\cdot}4\%$; silver proteinate gave $73{\cdot}1 \pm 6{\cdot}6\%$. In Fig. 1 there is plotted the result of a typical experiment with inulin.

The volume of fluid in which inulin was distributed was greater than that for the other agents. The variability of the data, however, prevents these differences from being highly significant. The difference of the means determined by inulin and by silver proteinate are at the borderline of significance with P of 0·04. The differences between the means of inulin and haemoglobin, and haemoglobin and silver proteinate are not statistically significant. The larger molecules may not penetrate into all of the spaces in which inulin is distributed. The pericardial sac and the lumen of the kidney are the most likely areas, but tests of the inulin content of the fluids of these spaces were not included while the work was in progress, and the animals are not now available for further tests.

Table 3. *Inulin, silver proteinate and haemoglobin volumes, total body water and cellular water of* Aplysia californicus

Animal wet wt. (g.)	Blood vol. (ml.)	Blood vol. as % wet wt.	Blood* water (ml.)	Dry wt. (g.)	Corrected† dry wt. (g.)	Body water as % wet wt.	Cellular water as % wet tissue wt.
				Inulin			
183·5	148	80·9	147	12·7	8·3	93·0	74·1
220·8	178	80·6	176	16·4	11·0	92·6	72·1
237·5	189	79·6	187	15·8	10·2	93·4	77·2
264·5	222	83·8	220	18·4	11·7	93·1	68·9
255·5	192	75·2	190	20·8	15·0	92·0	74·9
270·5	223	82·4	221	18·9	12·2	93·0	71·5
330·7	250	74·2	248	25·7	18·2	92·2	75·9
387·0	301	77·8	298	26·4	17·3	93·2	78·3
Mean	—	79·3	—	—	—	—	74·2
S.D.	—	3·1	—	—	—	—	3·0
				Silver proteinate			
146·0	91	62·3	90	12·7	10·0	91·4	81·3
184·0	144	78·3	143	14·0	9·1	92·4	73·7
236·0	163	69·0	161	16·4	11·5	93·0	83·6
236·5	188	79·5	186	17·0	11·3	92·8	74·7
267·0	200	75·0	198	19·9	13·9	92·5	78·0
286·0	174	62·0	172	23·4	17·8	91·7	82·1
308·8	232	75·1	230	27·8	20·8	91·0	71·1
330·7	256	76·0	253	25·6	18·0	92·3	74·3
333·7	269	80·6	266	24·1	16·1	92·8	73·0
Mean	—	73·1	—	—	—	—	76·9
S.D.	—	6·6	—	—	—	—	3·3
				Haemoglobin			
260·0	188	72·1	186	18·7	13·1	89·0	80·8
270·5	189	70·0	187	18·9	13·2	93·0	83·6
280·0	220	78·5	—	—	—	—	—
290·6	222	76·5	220	21·1	14·5	92·7	77·4
349·0	255	73·0	—	—	—	—	—
554·5	285	80·4	282	25·0	16·5	92·9	74·3
411·0	341	83·0	—	—	—	—	—
Mean	—	76·2	—	—	—	92·4	79·1
S.D.	—	4·4	—	—	—	1·0	3·5

* Corrected for the volume of the salt only.
† Corrected for the weight of the salt only.

Since *Aplysia* has a shell of negligible size it was relatively easy to obtain other data on each specimen of this mollusc. The mean of the total water content of the twenty-one animals was $92{\cdot}4 \pm 1{\cdot}0\%$. The mean of the percentage cellular water differed for inulin, silver proteinate and haemoglobin, the values being $74{\cdot}2 \pm 3{\cdot}0\%$, $76{\cdot}9 \pm 3{\cdot}3\%$, $79{\cdot}1 \pm 3{\cdot}5\%$, respectively

(*b*) Archidoris. The nudibranch *Archidoris* appears to have a more solid structure than *Aplysia*. This may be due in part to the nature of the integument, or perhaps to the body fluids being under greater hydrostatic pressure, for the percentage of total body water in *Archidoris*, $92{\cdot}4 \pm 1{\cdot}3\%$, is the same as that for *Aplysia*.

Table 4. *Inulin volume, total body water and cellular water of* Archidoris *sp.*

Animal wet wt. (g.)	Blood vol. (ml.)	Blood vol. as % wet wt.	Blood* water (ml.)	Dry wt. (g.)	Corrected† dry wt. (g.)	Body water as % wet wt.	Cellular water as % wet tissue wt.
50·6	28·6	56·4	28·3	4·22	3·36	91·7	84·3
51·2	32·3	63·1	32·0	3·44	2·47	93·1	86·5
53·1	30·3	57·1	30·0	3·43	2·52	93·5	88·7
54·6	38·4	70·3	38·0	5·03	3·88	90·8	74·9
57·8	42·5	73·6	42·1	3·85	2·58	93·2	82·1
62·1	44·8	72·2	44·4	4·50	3·15	92·0	80·7
Mean	—	65·5	—	—	—	92·4	82·9
S.D.	—	7·0	—	—	—	1·3	4·4

* Corrected for the volume occupied by the salt only.
† Corrected for the weight of the salt only.

By simple drainage a volume of blood approximately one-fourth the body weight could be obtained readily. This is much less than that from *Aplysia*, but more than that from other molluscs. Consequently, the relatively high percentage blood volume of $65{\cdot}5 \pm 7{\cdot}0\%$, was to be expected. The experimental data for this animal are presented in Table 4, and a typical experimental curve is given in Fig. 1.

The cellular water, $82{\cdot}9 \pm 4{\cdot}4\%$, is a higher value than was obtained for other forms reported, but may only represent a sampling error as the number of animals reported on is small.

(2) *Pulmonata*

(*a*) Achatina fulica. The giant African snail, *A. fulica*, appears to have an effective circulation since the equilibrium time was relatively short. The results of a typical experiment are given in Fig. 1, and the values of body weight, blood volume, etc., are given in Table 5. The blood volumes for eight animals was $40{\cdot}3 \pm 5{\cdot}6\%$, the body water $86{\cdot}4 \pm 3{\cdot}3\%$, and the cellular water $77{\cdot}1 \pm 5{\cdot}0\%$.

Although the animals used in the experiments were supplied with water, the possibility existed that the extent of hydration differed from animal to animal and

Table 5. *Inulin volume, total body water and cellular water of* Achatina fulica

Wet wt. without shell (g)	Blood vol. (ml.)	Blood vol. as % wet wt. without shell	Dry wt. (g.)	Body water as % wet wt. without shell	Cellular water as % wet tissue wt.	Blood protein (g./100 ml.)
41·0	15·8	38·6	6·90	83·2	72·6	0·60
44·5	15·8	35·5	7·10	84·0	75·2	1·63
45·1	23·5	52·2	4·80	89·5	77·8	0·55
45·8	18·5	40·4	8·00	82·4	70·8	0·15
62·3	28·6	45·8	6·95	89·0	79·4	0·36
69·8	24·6	35·2	10·05	85·7	77·7	—
71·8	25·0	34·8	5·50	92·4	88·2	—
73·8	28·6	39·8	11·45	84·6	74·8	—
Mean	—	40·3	—	86·4	77·1	0·45
S.D.	—	5·6	—	3·3	5·0	—

that this might account for some of the variability in the results. Animals which were kept in the laboratory with a restricted amount of water available lost about 10% of their weight the first day. However, the snails appeared to adjust to the desiccation and in some cases lost only about 20% of the original body weight after 9 days without water.

The range in total body water of the animals used in these experiments was about 10%. Nevertheless, the correlation coefficient, *r*, between percentage blood volume and percentage body water was only 0·26, a value which implies no direct relationship in the range tested.

The amount of protein in the blood of five animals was determined by a micro-Kjeldahl method and a mean of 0·46 g. of protein in 100 ml. of blood was obtained. The individual results are given in Table 5.

(*b*) Arion ater. A slug may lose as much as one-fifth of its initial wet weight during a 3–4 hr period. Therefore, care was taken during an experiment to keep the environment saturated with water and to minimize the amount of handling. To detect any changes in weight, the animals were weighed at the beginning and end of an experiment, and only the results for those animals which showed negligible weight losses (less than 5% of body weight) are reported in Table 6.

Table 6. *Inulin volume, total body water and cellular water of* Arion ater

Animal wet wt. (g.)	Blood vol. (ml.)	Blood vol. as % wet wt.	Dry wt. (g.)	Body water as % wet wt.	Cellular water as % wet tissue wt.
10·00	3·45	34·5	—	—	—
11·57	2·88	24·9	—	—	—
11·57	3·95	34·2	—	—	—
11·70	5·00	43·6	—	—	—
11·80	5·88	50·2	—	—	—
13·30	6·33	47·5	—	—	—
14·05	3·37	24·0	—	—	—
12·20	7·02	57·5	1·00	91·8	80·7
13·72	4·71	34·3	1·64	87·0	81·8
21·95	6·46	29·4	2·64	88·0	83·0
22·83	8·00	35·0	3·52	84·5	76·3
43·72	10·88	24·9	8·55	80·3	74·0
Mean	—	36·6	—	86·3	79·0
S.D.	—	10·4	—	3·8	3·4

There was a considerable amount of variability in the blood volumes obtained in the early experiments, the values ranging from 24·0 to 50·2 %. In order to ascertain whether this variability was attributable to the degree of hydration of the animal, dry-weight determinations were made in subsequent experiments. The coefficient of correlation, *r*, between blood volume and body water was found to be high at a value of 0·81 for the later experiments. However, due to the small number of experiments, this *r* is not reliable and the question is not settled.

The mean blood volume for all the animals reported was 36·6 ± 10·4%, the mean total body water was 86·3 ± 3·8%, and the cellular water 79·0 ± 3·4%. The variability in the results may not be extraordinary as the animal is terrestrial and does not possess a protective shell.

C. *Pelecypoda*

Filibranchiata

(*a*) Mytilus californianus. In *M. californianus* after the injection of the test material into the anterior aorta equilibrium was reached in a relatively short time. Data from a typical experiment are presented in Fig. 1, and the results of the blood-volume determinations, as well as other pertinent data, are given in Table 7. The average blood volume is $50{\cdot}8 \pm 7{\cdot}6\,\%$, the total body water is $88{\cdot}9 \pm 0{\cdot}36\,\%$, and the cell water is $79{\cdot}7 \pm 3{\cdot}6\,\%$.

Table 7. *Inulin volume, total body water and cellular water of* Mytilus californianus

Series A

1	2	3	4	5	6	7	8	9	10
Measured, wet wt. without shell (g.)	Measured blood vol. (ml.)	(2/1 × 100) blood vol. as % wet wt.	Measured dry wt. (g.)	(1 × mean of 13), dry wt. calculated from data of Series B (g.)	(1 × mean of 15), dry wt. corrected for blood salt (g.)	(2 − 1 % of 2), blood water* (ml.)	(1 − [5 + 7]), cellular water (ml.)	(8 + 6), wet cell wt. (g.)	(8/9 × 100), cellular water as % wet cell wt.
38·8	21·0	53·8	6·7	4·27	3·68	20·8	13·73	17·41	78·26
39·8	24·6	61·6	4·9	4·38	3·78	24·4	11·02	14·80	74·46
48·0	27·3	56·7	8·6	5·28	4·55	27·0	15·72	20·27	77·55
58·3	37·6	64·5	4·4	6·41	5·55	37·2	14·69	20·24	72·57
74·6	30·8	41·3	12·3	8·21	7·10	30·5	35·89	42·99	83·48
78·3	34·8	44·4	14·8	8·61	7·45	34·5	35·19	42·64	82·52
84·4	39·2	46·4	11·9	9·28	8·01	38·8	36·32	44·33	81·93
95·6	42·0	44·0	21·9	10·52	9·09	41·6	43·48	52·57	82·70
116·0	53·2	45·8	18·0	12·78	11·02	52·7	50·52	61·57	82·05
122·4	60·6	49·5	14·6	13·46	11·63	60·0	48·94	60·57	80·80
Mean	—	50·8	—	—	—	—	—	—	79·7
S.D.	—	7·6	—	—	—	—	—	—	3·56

Series B

11	12	13	14	15
Measured wet wt. without shell (g.)	Measured dry wt. (g.)	(12/11 × 100), dry wt. as % wet wt.	(12 − 0·03[11/2]), dry wt. without blood salt (g.)	(14/11 × 100), corrected dry wt. as % wet wt.
34·20	4·65	13·6	4·13	12·1
35·12	3·99	11·4	3·46	9·8
35·35	3·59	10·1	3·05	8·6
40·93	4·60	11·2	3·98	9·7
43·68	5·04	11·5	4·38	10·0
45·64	4·37	9·6	3·68	8·1
50·92	5·34	10·5	4·56	8·9
55·13	5·79	10·5	4·95	9·0
Mean 42·61	—	11·0	—	9·5

* Blood water is derived from blood volume on the assumptions that the blood protein of *Mytilus* is negligible (less than 0·1 %), and that the volume occupied by 3 g. of salt is 1 ml.
Dry weight is derived from measured dry weight by subtracting the salt content, assumed to be 3 %, of the blood estimated from Series A to be about 50 % of the wet body weight.

It will be noted that two series of experiments are presented. The blood-volume determinations were performed on animals recently brought to the laboratory, and work was completed before a decision had been reached to compute cellular water using a corrected dry weight. No attention had been paid to clearing the gut, a factor which is thought to account for the variability of the dry-weight measurements. Since the blood-volume measurements were technically quite demanding a new series was not performed. Instead, animals which had been kept in the

laboratory for several months were dissected and dried. The corrected dry weights obtained from these animals were then applied to the data derived from the first series. Errors are undoubtedly introduced by this process and it must be kept in mind that the values set forth for cellular water are not more than estimates.

(*b*) Margaritana margaritifera. In *M. margaritifera* the equilibration time was as short as 15 min. Generally, however, 30 min. elapsed before a linear relationship was established. This rapid equilibration may have been the result of the direct injection of the test material into the heart which insures the most rapid distribution through the circulatory system.

Table 8. *Inulin and T-1824 volumes, total body water and cellular water of* Margaritana margaritifera

Wet wt. without shell (g.)	Blood vol. (ml.)	Blood vol. as % wet wt. without shell	Dry wt. (g.)	Body water as % wet wt. without shell	Cellular water as % wet tissue wt.
31·4 (T-1824)	16·6	52·8	4·3	86·3	71·0
(Inulin	16·2	52·6	4·3	86·3	71·6
32·5 (inulin)	13·0	40·0	4·8	85·5	75·5
34·8 (inulin)	13·9	40·0	4·1	88·2	80·3
36·6 (T-1824)	19·0	51·9	4·7	87·2	73·3
(Inulin)	20·0	54·7	4·7	87·2	71·7
38·2 (inulin)	16·6	43·4	4·9	87·2	77·3
38·4 (inulin)	17·8	46·3	3·9	89·9	81·0
39·2 (T-1824)	24·4	62·2	—	—	—
(Inulin)	19·6	50·0	—	—	—
44·0 (inulin)	22·5	51·2	4·2	90·4	80·4
44·5 (inulin)	18·5	41·6	5·7	87·1	78·1
48·6 (inulin)	25·0	51·4	4·6	90·5	80·5
Mean	—	49·0	—	88·0	76·4
S.D.	—	6·0	—	1·5	3·8

The data obtained in a typical experiment are presented in Fig. 1, and the results for all the experiments reported are summarized in Table 8. The blood volume was found to be $49{\cdot}0 \pm 6{\cdot}0\%$. In three of the specimens, successful experiments were performed in which inulin and T-1824 bound to rabbit serum were injected simultaneously. Both substances appeared to measure the same space although they differ in chemical properties and the size of the molecule.

The volume of blood which could be drained from the visceral mass at the end of an experiment ranged from 30 to 50% of the volume determined by the dilution method. In a few experiments the concentration of inulin in the drained blood was determined in order to ascertain whether there was a significant contamination of the drained blood with water. In these cases the concentration of inulin in the drained blood was within the range of concentration of the experimental blood samples.

The total body water was relatively high, $88{\cdot}0 \pm 1{\cdot}5\%$, and the cellular water was $76{\cdot}4 \pm 3{\cdot}8\%$.

D. *Cephalopoda*

Octopoda

(*a*) Octopus hongkongensis. Most of the measurements of the blood volume of the octopus were made with T-1824. It was possible to use this material since the octopus has a high blood protein, about 10 g. of protein per 100 ml. of blood, to which the dye was bound. To substantiate the T-1824 estimate of the size of this compartment, colloidal HgS was also used as a test material. The two substances appear to measure the same fluid compartment, but differ in the rate of loss from the circulating fluid, the HgS being lost at the slower rate. Representative curves of experiments with these two substances are given in Fig. 1. In Table 9 are summarized the data for the octopus, the blood volume being $5{\cdot}8 \pm 1{\cdot}0\%$. The percentage total water was determined for three animals and was found to be 82·5% of the body weight. Using this value, the cell water is approximately 77% of the wet tissue weight.

Table 9. *Inulin volume, T-1824 volume and* HgS *volume of* Octopus hongkongensis

Animal wet wt. (g.)	Test material	Blood vol. (ml.)	Blood vol. as % wet wt.	Extracellular vol. (ml.)	Extracellular vol. as % wet wt.	Extracellular equilibration time (min.)
7,730	T-1824	588	7·6	—	—	—
4,550	T-1824	217	4·8	—	—	—
10,430	T-1824	624	6·0	—	—	—
6,360	T-1824	374	5·9	—	—	—
12,955	T-1824	759	5·9	—	—	—
22,500	T-1824, raffinose	1272	5·6	5460	24·3	165
8,640*	T-1824, inulin	492	5·7	1612	18·7	210
8,750	T-1824, inulin	492	5·6	1776	20·3	225
14,900	T-1824, inulin	884	5·9	4550	30·5	180
8,520	T-1824, inulin	649	7·6	3450	40·4	85
8,640*	HgS	374	4·3	—	—	—
14,780	HgS	943	6·4	—	—	—
8,175*	HgS, inulin	375	4·6	1835	22·5	125
19,550	Inulin	—	—	5970	30·6	195
4,890	Inulin	—	—	1775	36·5	120
Mean	—	—	5·8	—	28·0	—
S.D.	—	—	1·0	—	7·3	—

* Same animal.

In one experimental animal it was possible to make several measurements. In the first experiment, T-1824 as used with a resultant blood volume of 5·7%. Three days later, a repeat determination was made with colloidal HgS, and a blood volume of 4·3% was obtained. A month later, another measurement was made with HgS with a value of 4·6% being obtained. The animal seemed perfectly normal, the only change noted was a loss of weight from 8640 to 8175 g., although the animal had continued to eat regularly.

For comparison with the volumes obtained with T-1824 and HgS, the volume of

fluid in which inulin was distributed was measured. In some cases the inulin was administered simultaneously with the other agents, and at other times independently. The average value obtained for the inulin space was $28{\cdot}0 \pm 7{\cdot}3\%$. The experimental data are summarized in Table 9 and a typical experiment is shown in Fig. 1.

There has been anatomical evidence for a closed circulatory system in cephalopods, but experimental verification has been lacking. From the results that have been obtained, it is believed that inulin measures the total extracellular fluid, while T-1824 and HgS measure the blood volume. Since the inulin space was $28{\cdot}0\%$ and the blood volume $5{\cdot}8\%$, the tissue fluid compartment of the octopus is approximately 22% of the wet tissue weight.

The coefficient of correlation, r, between the time necessary for equilibrium and the size of the extracellular space was $-0{\cdot}71$. In testing the significance of this value a P of $0{\cdot}05$ was obtained even though the number of samples was small. Consequently, it appears likely that an inverse relationship exists between the two values.

DISCUSSION

The results of the determinations of blood volume in representative molluscs show a wide range of values within the phylum from $5{\cdot}8\%$ in *Octopus* to about 75% in *Aplysia*. Even if the total extracellular fluid of *Octopus*, about 28%, is considered to be the better figure for comparison with the representatives of the other classes, the range is still a very wide one and illustrates great diversity within the phylum.

The low blood volume of $5{\cdot}8\%$ reported for *Octopus* is obviously a different order of magnitude from any other of the forms studied. Only in this species was there a great difference in the volumes of fluid in which materials of different molecular size were distributed. The dye T-1824, which complexes with the protein of the blood and colloidal HgS (a material of colloidal particle size), appeared to measure the blood volume, while inulin distributed in a considerably larger space, probably the extracellular fluid. Since on a percentage basis the volume of the extra-cellular fluid of the *Octopus* is lower than the blood volumes of the other forms, it follows that a closed circulatory system containing a high concentration of respiratory pigment separated from a tissue fluid compartment may meet greater metabolic demands than an open circulatory system with a larger total volume of fluid.

Robertson (1953) reported a value of 33% for the extracellular fluid of the cephalopod *Sepia*, but with the reservation that this might be an overestimate due to the possible entrance of the test material, sucrose, into muscle cells. It was noted in the course of the work reported here that there was an inverse relationship between the size of the extracellular space measured by inulin and the time required for equilibration. This observation would appear to indicate differences in permeability of tissues from animal to animal, so that a high permeability and a rapid attainment of equilibrium would also permit the entrance of inulin into other spaces, and a higher apparent extracellular fluid volume. A part of the variability of the results with this substance may rest on these unexplained differences in permeability. Penetration of inulin into other spaces will be discussed later in this paper.

The groups which show total blood volumes next larger in size to those of the

cephalopods are the pulmonate gastropods. Since both *Achatina* and *Arion* have open circulatory systems and are terrestrial, the differences expected between their average blood volumes, 40·3 and 36·6%, respectively, would not be very great. When the *t* test was applied to the data for these forms, it was found that the difference was not large enough to be statistically significant given the variability encountered in the measurements.

The mean blood volume of 44% obtained for the most primitive of the animals included in this study, *Cryptochiton*, is somewhat larger than those of the pulmonates. This value is statistically significantly different from that of *Arion*, but not from that of *Achatina*.

In order of increasing blood volume, the next species encountered are in the class Pelecypoda. *Mytilus*, with a blood volume of 51%, and *Margaritana* at 49%, show a striking similarity in spite of one being a marine and the other a fresh-water animal. Since the animals are similar in size, shape and morphology, perhaps this should be expected. The difference between the means of these two forms is not statistically reliable, but the blood volumes of these animals are significantly different from all the others reported here.

The results obtained with *Mytilus* and *Margaritana* confirm the findings of Potts (1954) who determined the inulin space of the fresh-water pelecypod *Anodonta cygnea* to be 55% of the wet weight without shell. In view of this similarity in blood volume of one marine and two fresh-water pelecypods, and the general agreement of these results with those on the other molluscan forms, it appears likely that the value of 8% of the wet tissue weight reported for two other fresh-water pelecypods by Prosser & Weinstein (1950) may be too low. Low values might be obtained under a number of different circumstances. Because they injected through a hole in the shell, without visual confirmation that the tip of the needle lay within the heart, it is possible that the material was injected into some fluid other than the circulating blood. On the other hand, we have noted that too rapid an injection of test material into the circulatory system resulted in impairment of heart action. It is possible that in their experiments the injected material entered the blood, but was mixed with a relatively small part of the total volume of the circulatory system in the 30 min. of the experiment. As confirmatory evidence for the low value Prosser & Weinstein (1950) cite the recovery from the heart at the conclusion of the experiment of fluid amounting to 96% of the volume measured by T-1824. In none of the species studied in our work was it possible to recover quantities of this order of magnitude, and it is our opinion that such a recovery is rather an indication of error than a satisfactory accounting for the volume of blood of the animal.

The largest blood volumes were found in the representatives of the opisthobranchs, *Archidoris* having a mean value of 65% and *Aplysia* one of approximately 75%. The difference between the means is statistically significant and the blood volumes of these animals are reliably different from those of all the other animals investigated. These blood volumes are so large that it must be inferred that the blood is meeting not only the ordinary demands upon a circulatory system, but some other requirement of these animals. The idea that water may serve as a

structural element goes back at least to the arguments for the gonocoel theory advanced by Hatshek (Goodrich, 1945). The representatives of the group studied in this work demonstrated a very definite control over the distribution of the blood in the body. It will be recalled that until a multiply perforated tube was inserted in the haemocoel for more than half the body length every opening in it could be closed off by contraction of the animal's body wall. The animal demonstrated fine control of its body form in other respects. A large *Aplysia* depleted of much of its supply of blood soon took on a normal form and could be recognized by inspection as having been a larger animal only by the relatively large size of the ctenidium. It may also be pointed out that this group feeds on marine plants which are relatively voluminous for the amount of dry digestible nutrients. On this poor diet, a large body size would be perhaps of competitive value, and a large volume of water would represent a cheap way of attaining size without increasing the metabolic requirements exorbitantly.

The differences in blood volume obtained with various substances in *Aplysia* may serve to illustrate the statement of Potts (1954) that it is likely that inulin determines not only the circulating blood volume, but also the pericardial and renal coelomic space in *Anodonta*. Measurements with silver proteinate gave 73%, with inulin, 79%—a difference which amounts to 6% of the wet body weight. This might be due to failure of the silver proteinate to penetrate into these spaces. The relative size of this error would then vary from species to species, depending upon the size of these spaces compared to the blood volume.

Further evidence for the penetration of inulin into other spaces is found in the experiments on excretion in the *Octopus* (Harrison, 1954) and in *Achatina fulica* (Martin, Harrison & Stewart, 1953). In these forms it is known that inulin penetrates the renal space, and appears after a lag period in the urine in approximately the same concentration as that found in the blood. Robinson & McCance (1952) have stated well the difficulties in using single injections of inulin in mammals when excretion is rapid. In contrast, the excretion by molluscs is very slow. Loss of inulin by this route may be compensated by the extrapolation of the dilution curve back to the time of injection. However, the entrance of inulin into the pericardial and renal spaces may account in part for the early, non-linear aspect of the dilution curve. In view of all the evidence at hand it may be well to regard all the volumes measured with inulin as somewhat greater than the true blood volume.

In addition to the differences in blood volume from species to species, there was also a considerable variation in the amount of time necessary for equilibration. This is probably due to a number of factors, including the region of injection, the effectiveness of the circulatory system, and the amount of movement of the body. In an inactive form such as *Cryptochiton*, the injection had to be made into the haemocoel, and slow equilibration might be expected. In contrast, when the injection was made directly into the heart and the animal showed considerable bodily activity, as in *Margaritana*, the equilibration would be rapid. Slow equilibration made necessary a longer extrapolation with a consequent multiplication of any errors due to differences in permeability.

Another aspect of the data is yet to be considered. The total body water ranged in value from 82·5 to 92·4%. The *t* test was used to determine the significance of the differences in the mean total water contents. The mean body water of *Aplysia* and *Archidoris*, both 92·4%, is reliably different from all of the others. The difference between *Mytilus* at 84·6% and *Octopus* at 82·5%, is significant, while that between *Mytilus* and *Margaritana*, 88%, is probably significant—$P = 0{\cdot}02$. None of the remaining comparisons was reliably different.

The parameter to be considered in conclusion is the cellular water. For each species a computation of the percentage of cellular water has been made and the data presented in the tables for individual animals. This computation can be obtained readily, since for most molluscs there is an open circulatory system and the shell, if present, is easily freed from the body tissues.

The range of values of cellular water is from 76·4 to 82·9% and differences between the values are hardly significant. Possible sources of error have been pointed out to which it should be added that no correction was applied to the data for the connective tissue of the animals. Even though these values should be considered only as approximations, it is of interest to compare them with some of those reported in the literature. The water content of the tissues of molluscs is not well known. Balland's (1898) values are those generally cited, but this author made no effort to distinguish extracellular from intracellular water, and other reports on molluscan tissue suffer from the same difficulty. On the other hand, Ephrussi (1933), working with echinoderm eggs and larvae, corrected for extracellular water in his determinations. In 40 hr. larvae of *Paracentrotus lividus* he found the cellular water amounted to 78·8% of the total wet weight. In the vertebrate group of animals it is only in recent years that the results of computations of the water content of mammalian cells have been in general agreement from laboratory to laboratory. Chanutin & Ludewig (1939) calculated the intracellular water of rat muscle fibres to be 73·2%. Lowry & Hastings (1942) are in essential agreement, their value being 74%. These figures are further confirmed by a 74·2% value for dog muscles (Muntwyler, Mellors, Mautz & Mangun, 1940) and of 75·1% for cat muscle which can be calculated from the data of Crismon, Crismon, Calabresi & Darrow (1943). But most of these values rest on the debatable assumption that there is no chloride in the cells and so the values may also be considered to be estimates. Computations for certain other mammalian tissues are available, but since these computations only demonstrate the expected variation from mammalian tissue to tissue, they will not be cited here. The water content of the various living cells reported here is, as might be expected, quite comparable with the others.

One of the objectives of this study was to examine the normal variability in blood volume in representatives of this phylum of animals. A number of factors have been discussed which may be sources of error in the results. However, within one species, these errors should be reasonably consistent, and the range of variability of blood volume is thought not to be due entirely to technical faults, but as reflecting the inability of these animals to regulate their blood volume with a high degree of stability.

SUMMARY

1. Individuals of *Cryptochiton stelleri*, as representatives of the class Placophora, distributed inulin in blood volumes yielding a mean of 43·8% of the wet body weight without shell. Mean cellular water was estimated to be 76%.

2. The two opisthobranch gastropods examined were found to have very large mean blood volumes. *Aplysia californicus* distributed haemoglobin in 76·2% and mild silver proteinate in 73·1% of the wet body weight. Inulin was distributed in a significantly larger space averaging 79·3% of the body weight, which probably included pericardial and renal spaces. *Archidoris* sp. distributed inulin in 65·4% of the wet body weight. Mean cellular waters were 74–79% in *Aplysia* and 83% in *Archidoris*.

3. Two pulmonate gastropods were studied with inulin which was distributed in a mean space 40·3% of the wet body weight without shell of *Achatina fulica*, and 36·6% of the wet body weight in *Arion ater*. The computed cellular waters were 77 and 79%, respectively.

4. Excellent agreement was shown between a marine pelecypod, *Mytilus californianus*, and a fresh-water pelecypod, *Margaritana margaritifera*, of similar body size and form. Inulin in the former was distributed in 50·8%, and in the latter inulin and T-1824 in 49% of the wet body weight without shell. The cellular water contents were 80 and 76%, respectively.

5. In a single representative of the Cephalopoda—*Octopus hongkongensis*, it was possible to demonstrate with T-1824 and with HgS a blood volume averaging 5·8% of the wet body weight, constituting a fluid space distinctly different from the tissue fluid space. Inulin was distributed in the entire extracellular space amounting to 28% of the wet body weight. The cell water which was calculated from the mean values obtained was 77%.

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THE UPTAKE OF RADIOACTIVE CALCIUM BY SEA URCHIN EGGS. I. ENTRANCE OF Ca^{45} INTO UNFERTILIZED EGG CYTOPLASM

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THE UPTAKE OF RADIOACTIVE CALCIUM BY SEA URCHIN EGGS. I. ENTRANCE OF Ca^{45} INTO UNFERTILIZED EGG CYTOPLASM [1]

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In studying the physiology of calcium accumulation of sea urchin eggs and its ultimate utilization in the formation of the calcareous skeleton, the use of radioactive calcium as a tracer offers many advantages. But a search of the literature on the uptake of radiocalcium by sea urchin eggs revealed only one report which was a study using an indirect method (Rudenberg, 1953). In his study of the role of the jelly coat in the uptake of calcium by the eggs of *Arbacia punctulata* Rudenberg observed the radioactivity present in his samples by a Geiger tube placed above the incubation medium. He concluded that Ca^{45} was accumulated only by eggs with jelly coats and for up to six hours after fertilization. About six hours after fertilization a loss of calcium from the eggs was noted. But eggs without jelly coats showed no uptake and no loss of calcium. In the present study, experiments have been designed to give more direct answers to the following series of questions: (1) Will sea urchin eggs, with and without jelly coats, take up radiocalcium from the medium? (2) If either or both types of eggs take up radiocalcium from the medium, are the Ca^{45} ions simply adsorbed on the surface or can they be demonstrated in the egg cytoplasm? (3) If there is an uptake of radiocalcium, is it due to a net accumulation of calcium or simply to an exchange of Ca^{45} with stable Ca^{40}?

Materials and Methods

Mature, unfertilized eggs of *Tripneustes gratilla* (Linnaeus), a large, common species of sea urchin in Hawaiian waters, were used. Freshly collected females were induced to shed their eggs by the KCl injection method (Tyler, 1949). One female could provide 30–50 ml. of eggs, more than enough for a whole series of experiments. The eggs were strained through bolting cloth or several layers of cheesecloth to remove extraneous matter, and washed with filtered sea water with the help of gentle centrifugation before use in the following experiments.

(1) *Comparison of Ca^{45} uptake by intact eggs and by eggs without jelly coats.* To compare the time course of the uptake of radiocalcium by these two types of eggs, a freshly washed batch of eggs from one female was divided into two approximately equal aliquots. One aliquot was treated with HCl to remove the jelly coats by the method of Harvey (1941). A stock Ca^{45} solution with an activity of 5 μc/ml. was made by dilution of Oak Ridge $Ca^{45}Cl_2$ solution having a specific activity of 73.81 mc/g. with an appropriate quantity of filtered normal sea water. One-half ml. of a 1:10 suspension of eggs with jelly coats removed was put into a 50-ml.

[1] Contribution no. 100 from Hawaii Marine Laboratory, University of Hawaii.

beaker already containing 4 ml. of filtered sea water. One-half ml. of the stock Ca^{45} solution was then added, giving a final volume of 5 ml. and a Ca^{45} concentration of 0.5 μc/ml. Twelve beakers were similarly prepared. The content of the first to the twelfth beaker was incubated at room temperature (24–27° C.) with occasional stirring according to the following schedule:

Beaker No.	1	2	3	4	5	6	7	8	9	10	11	12
Incubation time in minutes	0	1	2	4	8	16	32	64	128	256	512	1024

At the end of the specified time of incubation, the content of each beaker was centrifuged to separate the eggs from the incubation medium. As it took two minutes to effect this separation, the actual time of contact between the eggs and Ca^{45} varied from 2 to 1026 minutes.

A 0.5-ml. aliquot of the supernatant medium after each time interval was transferred to an aluminum planchette, dried and counted with a thin window (1.4 mg. cm^2) Geiger tube and a commercial scaler. All samples were prepared in triplicate. Dose planchettes were made of the incubation medium without eggs by diluting it ten-fold and triplicate of 0.5-ml. volume used for counting. A convenient aliquot of the incubated eggs from each time interval was transferred from the centrifuge tube, spread on a weighed planchette, dried to constant weight and the activity of the eggs counted in the same way as the medium. After correction for background and for self-absorption the counts were expressed in counts per minute per mg. dry weight. A similar series was run using eggs with jelly coats intact. These two experiments were repeated, but the incubated eggs were washed in normal filtered sea water before being counted for Ca^{45} activity. As a control, eggs from the same female were incubated in normal filtered sea water and prepared and counted in a similar manner.

(2) *Removal of Ca^{45} from eggs.* In order to ascertain whether the activity of eggs incubated in radiocalcium-containing sea water could be removed from the egg surface, radioactive eggs, with and without jelly coats, were washed with (a) sea water, (b) a 0.02 per cent V/V solution in sea water of a commercial surface active wetting agent, "Sterox SK" (supplied by Monsanto Chemical Co.), and (c) a 0.05 per cent W/V solution in sea water of a commercial detergent "Tide," manufactured by Procter and Gamble Co. Samples of eggs without jelly coats and of intact eggs were washed separately by re-suspending the radioactive eggs in 10 ml. of the washing fluid in a centrifuge tube, and stirring them gently for 5 minutes. At the end of 5 minutes a 0.5-ml. aliquot of the well-mixed egg suspension was transferred to a second tube, centrifuged to remove the washing liquid from the eggs which were then spread on a planchette in order to measure egg radioactivity. The 9.5-ml. egg suspension in the original tube was centrifuged at a RCF of 2330 G to pack down the eggs. The sedimented eggs were completely drained by inverting the tube whose inside wall was wiped dry with absorbent tissue. The washing procedure was repeated five times with each sample of eggs.

(3) *Autoradiography of eggs.* Intact eggs and eggs without jelly coats were made radioactive by incubating them in sea water containing Ca^{45}. They were fixed separately with neutral formalin, dehydrated by direct transfer into dioxane (diethylene dioxide) which was changed once. Infiltration was made with two changes of paraffin. Sections of eggs 5 or 10 microns thick were mounted on slides

from some of which the paraffin was removed by xylene, and the remaining material coated with a thin veneer of gelatin and autoradiographs made on Kodak medical (No Screen) x-ray films. Other sections were treated by Townsley's method (personal communication). In this method, which is similar to that worked out by Bélanger and Leblond (1946), the paraffin sections were floated on slides previously coated with gelatin (Kodak photographic inactive gelatin, 0.5% and chrome alum, 0.05% solution) and allowed to attach by drying. After dissolving the paraffin in xylene the sections were run down through grades of alcohol to double glass-distilled water. The slides were next coated with Ilford nuclear research emulsion, type G.5, which was warmed to 38° C., thinned with a little distilled water and applied in a very thin layer with an artist's brush. The coated slides were dried under an electric fan and placed in a slide box made light proof by being wrapped in aluminum foil and black paper. Exposure of the emulsion to the β-radiation from egg sections was completed in a refrigerator and later developed photographically. Examination of the autoradiographs was made with both light and phase contrast microscopes.

(4) *Total calcium determination.* The total calcium content of ordinary and radiocalcium-containing eggs was estimated after the eggs were dried to constant weight. Aliquots of the dried specimens were wet-ashed by the method of Norris and Lawrence (1953). The egg calcium was precipitated with ammonium oxalate according to Holth's (1949) recommendation to exclude magnesium. The precipitated calcium oxalate was quantitatively estimated by flame spectrophotometry with a Beckman model DU spectrophotometer according to the method published in "Application Data for Beckman Instruments" by the manufacturer. A series of eggs having various radioactivities, from zero to 686 cpm per mg. dry weight, was used for analysis.

Results

When exposed to Ca^{45} in the medium eggs with and without jelly coats showed high rates of radiocalcium uptake during the first few minutes. After this initial phase the quantity of Ca^{45} taken up by the eggs increased with the increased time of incubation. Figure 1 represents the results of a typical experiment. Three things are shown by this figure: (1) unfertilized eggs, with and without jelly coats, took up radiocalcium; (2) unfertilized eggs without jelly coats took up more radiocalcium than intact eggs when both were treated similarly, as shown by the heights of curves A and C in contrast to those of curves B and D of this figure. This was true throughout the whole time course at all the different time intervals of incubation used. (3) When the eggs were washed with filtered sea water after incubation and before being placed on the planchettes for counting, both types of eggs retained Ca^{45}, but the activity due to Ca^{45} retained by eggs without jelly coats was much higher than that of intact eggs, as shown by curves C and D. Eggs from the same female but incubated in normal sea water as control showed no radioactivity at all.

Table I shows the results of washing the treated eggs in order to see if the Ca^{45} taken up by the two types of unfertilized eggs can be removed, assuming that the radiocalcium ions are on the surface of the jelly or in the jelly coats in the case of intact eggs, and on the surface of the vitelline membrane in the case of eggs

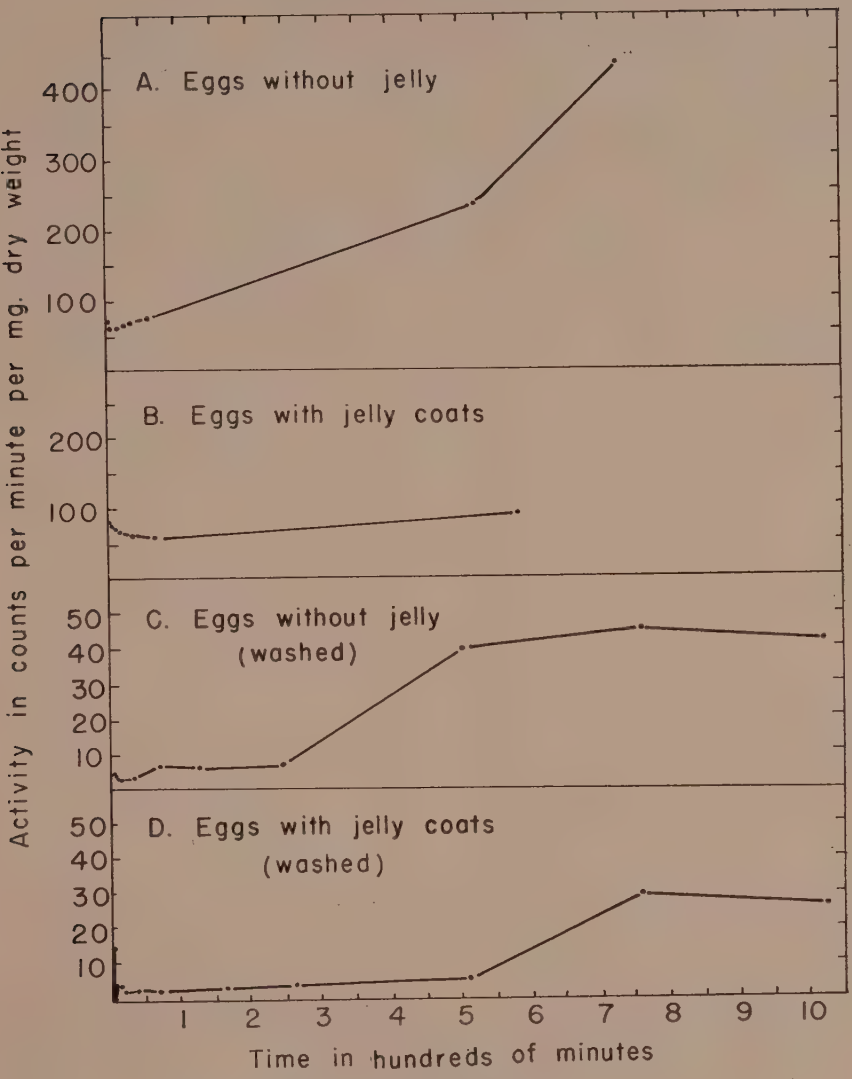

FIGURE 1. Radiocalcium uptake by sea urchin eggs with and without jelly coats for different periods of incubation.

without jelly. It will be seen from this table that eggs without jelly coats had approximately 1.5 times as much activity as intact eggs. Intact eggs washed once with detergent for five minutes lost about 80 per cent of the original radioactivity. On the other hand, eggs without jelly similarly treated lost only about half of the

original activity. After the fourth wash the washing fluid showed practically no activity, indicating that a surface active agent could not remove any more Ca^{45} from these eggs. But it will be noticed from Figure 2 that in washing with the wetting agent "Sterox SK," eggs without jelly coats consistently retained more radioactivity after each washing than did eggs with jelly coats. At the end of the fifth washing eggs without jelly coats retained 11 times as much activity as did similarly treated intact eggs, while the washing fluid showed no activity in either case. In washing with a solution of "Tide" (see Table I) about six times as much activity was retained by jelly-free eggs as by eggs with jelly coats. It was noticed during these washing experiments that the first washing of intact eggs removed all the jelly coats.

That the activity remaining in the detergent washed eggs was due to Ca^{45} in the cytoplasm and not removable by surface active agents is shown by autoradio-

TABLE I

The loss of radiocalcium from eggs washed with detergents showing that eggs without jelly coats retained more Ca^{45} than did intact eggs

Material	Washing fluid	Washing No.	Egg activity, cpm	Material	Washing fluid	Washing No.	Egg activity, cpm
Eggs with jelly coats intact	"Wetting agent" 6 drops, sea water 10 ml.	0	499	Eggs without jelly	"Wetting agent" 6 drops, sea water 10 ml.	0	736
		1st	103			1st	321
		2nd	34			2nd	137
		3rd	34			3rd	83
		4th	33			4th	80
		5th	5			5th	56
Eggs with jelly coats intact	"Tide" 1% w/v solution 0.5 ml., sea water 9.5 ml.	0	499	Eggs without jelly	"Tide" 1% w/v solution 0.5 ml., sea water 9.5 ml.	0	736
		1st	121			1st	306
		2nd	31			2nd	199
		3rd	173(?)			3rd	81
		4th	46			4th	82
		5th	9			5th	52

graphs of sectioned eggs. In Figure 3 are shown photomicrographs of some of the autoradiographs. Figure 3A is a photomicrograph of an autoradiograph made by three whole unfertilized eggs on an Ilford emulsion. The dark regions around these eggs show the presence of Ca^{45} in the jelly coats. Figure 3B shows an autoradiograph made on x-ray film by an intact egg incubated in Ca^{45}-containing sea water. In this figure the outline of the jelly coat and the egg can be seen. Figure 3C is a lower power photomicrograph made with a light microscope. In the center of this photomicrograph is a section of an untreated egg included among treated eggs whose sections show the presence of Ca^{45} inside the cytoplasm. Figures 3D–3F are photomicrographs made with a phase contrast microscope. In Figure 3D the egg membrane and cytoplasm are seen to contain radiocalcium. Figure 3E is a higher power photomicrograph of the autoradiograph of another group of eggs, while Figure 3F is a detailed view of a single section of a radioactive egg.

The results of chemical estimation of total calcium content of eggs having taken

up various quantities of radiocalcium are shown in Figure 4. This figure shows that of the 15 groups of radioactive eggs analyzed, no group showed significantly different content of total calcium from the average value of all the groups, although the radioactivity of the highest group was about 340 times that of the lowest. It also shows that increase in Ca^{45} taken into the eggs was not followed by increase

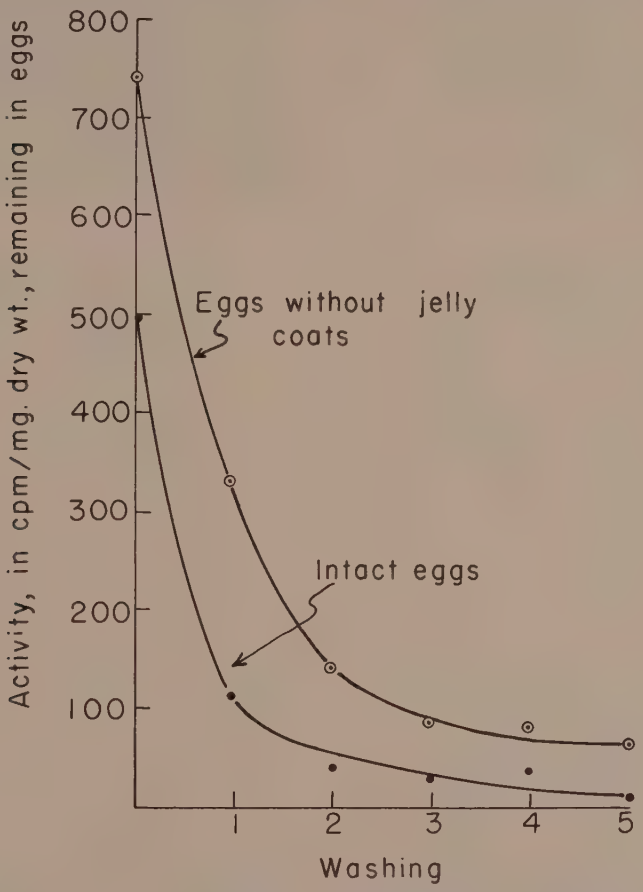

FIGURE 2. Decrease in radiocalcium in sea urchin eggs after washing with detergent solution (Sterox SK).

in total calcium. Total calcium fluctuated independently of the Ca^{45} content of the eggs about a mean value of 2.7 μg per mg. dry weight. The calcium content of ordinary, *i.e.*, unincubated, eggs was not lower than that of radioactive eggs. This indicates that there is no calcium accumulation, but an exchange of ordinary Ca^{40} for radioactive Ca^{45} leaving the total calcium content more or less unaltered.

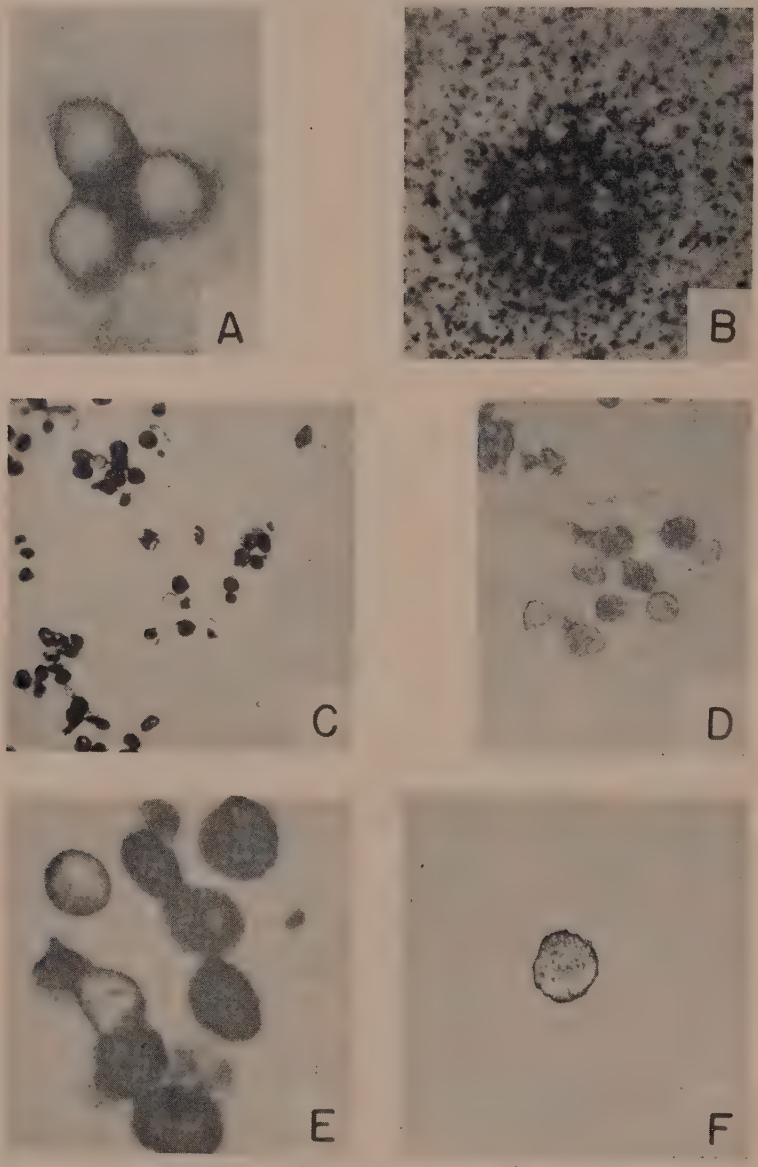

FIGURE 3. Photomicrographs showing Ca^{45} inside egg jelly and in sections of egg cytoplasm. 3A, autoradiograph of three intact eggs showing Ca^{45} among the jelly coats, magnification 100 ×. 3B, autoradiograph on Kodak x-ray film of one intact egg, magnification 150 ×. 3C, autoradiograph of 10 μ thick sections of eggs, magnification 37 ×. 3D, phase contrast photomicrograph of similar egg sections, magnification 70 ×. 3E similar to 3D, magnification 140 ×. 3F, detailed picture of a single section of egg, magnification 140 ×.

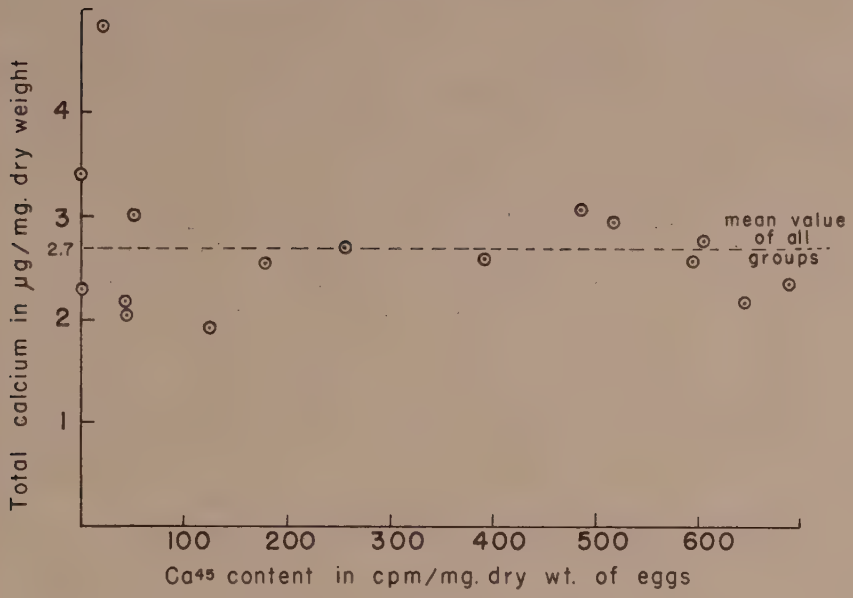

FIGURE 4. Total calcium content of ordinary and radioactive eggs showing no increase in total calcium with increase of Ca^{45} uptake.

DISCUSSION

The consistently greater amount of Ca^{45} taken up by eggs without jelly coats than that by intact eggs indicates that not only is jelly coat unnecessary for the uptake of radiocalcium, but it acts as a hindrance to the entrance of these ions into the egg cytoplasm. As the total diameter of intact eggs, inclusive of jelly coat, measures 126–130 microns, and that of the vitellus about 82 microns, an ion on the surface of the jelly coat would theoretically have to travel 63–65 microns to reach the geometrical center of the egg, while in the case of eggs without jelly, an ion on the surface of the vitelline membrane would have to travel only a distance of 41 microns to reach the same point. It is hence not surprising that for an equal period of incubation in sea water containing radiocalcium, eggs without jelly could take up more Ca^{45} than intact eggs. In other words, in eggs without jelly coats the Ca^{40} of the egg cytoplasm could be exchanged directly with the Ca^{45} in the incubation medium or on the vitelline surface. In intact eggs, the first place of Ca^{45} entry would be the jelly where the entering Ca^{45} could either exchange with the Ca^{40} of the jelly coats or continue to move into the vitellus.

When eggs with jelly coats were washed with detergent, the first washing removed most, if not all, of the jelly. If these eggs were first made radioactive, approximately 80 per cent of the radioactivity came off with the jelly. This observation suggests a possible explanation of the loss of calcium by fertilized *Arbacia* eggs after 6 hours incubation reported by Rudenberg (1953). As the intact radioactive eggs were shaken for 6 or more hours, a good deal of the jelly coats would

be detached which, when brought to the surface by stirring, would account for the reported increase in radioactivity in the surface layer of the incubation medium.

Although the autoradiographs of sections of the radioactive eggs do not give the exact location of the Ca^{45} ions in the cytoplasm, because the denaturation of the egg protein by formaldehyde fixation and subsequent histological procedures applied to the eggs and their sections may have altered their positions, the fact that these histological treatments and photographic developments used in connection with Ilford emulsion film coatings did not remove them indicates that the Ca^{45} must be firmly attached to the organic compounds of the eggs. An approximate idea of the site of the uptaken Ca^{45} is provided by the autoradiographs. Further investigations are in progress to determine which fraction or fractions of the egg substance combined with the radiocalcium.

Summary and Conclusions

1. The entrance of radiocalcium into unfertilized eggs of *Tripneustes gratilla* (Linnaeus) has been investigated in this study. By direct measurements of the radioactivity of intact eggs and of eggs without jelly coats it was found that although both types of eggs took up Ca^{45}, eggs without jelly coats took up much more than intact eggs.

2. By washing with detergent both types of eggs made radioactive by incubation in radiocalcium-containing sea water, it was found that jelly-free eggs retained 11 times as much Ca^{45} as intact eggs when washed with a commercial wetting agent "Sterox SK," and 6 times as much when washed with a dilute solution of "Tide."

3. It is concluded that jelly is not essential to the uptake of radiocalcium by sea urchin eggs and the presence of jelly coats reduces the amount of penetration into the egg cytoplasm.

4. Autoradiographs of whole eggs showed the presence of Ca^{45} in the jelly coats if intact eggs were incubated in radiocalcium-containing sea water. Autoradiographs of sections of both types of eggs showed that Ca^{45} was inside the egg cytoplasm.

5. No significant difference in total calcium was found by chemical analysis of groups of eggs incubated for various periods of time in Ca^{45}-containing sea water, *i.e.*, made to take up varying amounts of radiocalcium. None of the groups showed significantly different contents of total calcium from that of ordinary unfertilized eggs. It is concluded that Ca^{45} enters the egg cytoplasm by exchange with Ca^{40} of the eggs.

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THE LIFE HISTORIES OF SOME HAWAIIAN HETEROPHYID TREMATODES*

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INTRODUCTION

In March 1956 the author was flown to Hawaii by the United States Navy. With the assistance of Dr. and Mrs. A. H. Banner and Ralph Hinegardner, several thousand snails, *Stenomelania newcombi* Lea and *Tarebia granifera* Lam., were collected in ditches surrounding taro patches in Kaneohe, Oahu. They were taken to the Hancock Foundation, University of Southern California, for examination and life cycle experiments which revealed that 4 species of heterophyid trematodes, *Centrocestus formosanus* (Nishigori, 1924), *Haplorchis taichui* (Nishigori, 1924), *H. yokogawai* (Katsuta, 1932), and *Stellantchasmus falcatus* Onji and Nishio, 1924, use one or both of these snails as first intermediate hosts. The work was completed at the Marine Laboratory, University of Hawaii.

Very little information has been published on Hawaiian heterophyids even though the infections are common in *S. newcombi* and *T. granifera,* at least one of the second intermediate hosts, *Mugil cephalus,* is eaten raw by humans, and 2 cases of human infection have been reported (Alicata and Schattenburg, 1938; Glover and Alicata, 1957).

Centrocestus formosanus was described by Nishigori (1924a) and placed by him in the genus *Stamnosoma* which was erected by Tanabe (1922) but is synonymous with *Centrocestus* Looss, 1899 (Chapin, 1926). Nishigori stated that the first intermediate host was a species of *Melania* into which the miracidium penetrated and developed into a redia. He did not mention a sporocyst stage. The rediae produced cercariae which encysted in the gills of fresh water fish. He listed the following second intermediate hosts: *Carassius auratus* (Linnaeus), *Clarias fuscus* (Lacépède), *Channa formosana* (Jordan and Evermann), *Pseudorasbora parva* (Schlegel), *Rhodeus ocellatus* (Kner), *Gambusia affinis* (Baird and Girard), *Polyacanthus operculatus* (Linnaeus), *Ctenopharyngodon idellus* (C. and V.), *Ophiocephalus tadianus* (Jordan and Evermann), *Misgurnus anguillicaudatus* (Cantor), *Parasilurus asotus* (L.), *Zacco platypus* (T. and S.) and *Cyprinus carpio* (L.). He found the night heron, *Nycticorax nycticorax* (L.) to be a natural definitive host and obtained experimental infections in several species of laboratory animals and in man. Vazquez-Colet and Africa (1938, 1939, 1940) added to the list of second intermediate hosts, *Ophiocephalus striatus* Bloch, *Glossogobius giurnus* Buchanan-Hamilton, *Therapon plumbeus* (Kner), *Anabas testudineus* (Bloch)

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Contribution No. 101, Hawaii Marine Laboratory, University of Hawaii.

and *Hemiramphus dussumieri* Cuvier and Valenciennes from Phillippine waters. Chen (1942, 1948) provided additional information on the morphology and life cycle of this parasite including the fact that a frog, *Rana limnocharis* Boie and a toad, *Bufo melanostictus* Schneider, could serve as second intermediate hosts. However, attempts to parasitize adult frogs were negative. Therefore, he concluded that the tadpole stage must become infected (Chen, 1948). He found that *Carassius auratus, Macropodus opercularis* (L.), *Puntius semifasciolatus* (Gunther), *Limia caudofasciata* Regon and *Misgurnus anguillicaudatus* could serve as second intermediate hosts in China.

Haplorchis taichui was described by Nishigori (1924b) and placed in the genus *Monorchotrema. Monorchotrema* was declared a synonym of *Haplorchis* Looss, 1899 by Witenberg (1930). The life cycle of *Haplorchis taichui* was first outlined by Nishigori (1924b), then traced in detail by Faust and Nishigori (1926). Hsu (1950, 1951) also made a detailed study of the life cycle of this and related species and showed that Faust and Nishigori had incorrectly described the tail fins of the cercaria. The first intermediate hosts were species of the snail genus *Melania, M. obliquegranosa* (acc. to Faust and Nishigori) and *M. tuberculata chinensis* (acc. to Hsu). Second intermediate hosts included: *Cyprinus carpio, Carassius auratus, Zacco platypus, Pseudorasbora parva, Rhodeus ocellatus, Gambusia affinis, Ctenopharyngodon idellus* (acc. to Faust and Nishigori, 1926); *Varicorhinus* sp., *Tilapia simonis, Barbus canis* and *Gambusia affinis* (acc. to Witenberg, 1929); *Ophiocephalus striatus* (acc. to Vazquez-Colet and Africa, 1939); and *Oryzia latipes* and *Macropodus opercularis* (acc. to Hsu, 1951). Definitive hosts included: birds, *Nyctycorax nyctycorax* (acc. to Nishigori, 1924b), cattle egrets (Africa, 1938), *Larus* sp. (Witenberg, 1929), ducklings (Hsu, 1951) and mammals, cats and/or dogs (Faust and Nishigori, 1926; Witenberg, 1929; Africa and Garcia, 1935; Africa, 1938; Kuntz and Chandler, 1956); fox, *Vulpes vulpes* (acc. to Kuntz and Chandler, 1956); and man (Faust and Nishigori, 1926; Africa and Garcia, 1935; Africa, 1938; and Africa, de Leon and Garcia, 1935ab, 1936ab, 1937, 1940). This species has been reported from China (Chen, 1936), Egypt, (Kuntz and Chandler, 1956), Formosa (Faust and Nishigori, 1926), India (Bhalerao, 1936), Israel (Witenberg, 1929), and the Philippines (Vazquez-Colet and Africa, 1939).

Haplorchis yokogawai was described by Katsuta (1932a) from Formosa. He found the mullet to be a second intermediate host. Chen (1936) added further details of morphology especially of the gonotyl of specimens collected in China. Africa and Garcia (1935) and Africa (1938) reported this species from man in the Philippines. Chen (1936) reported this species from China and Bhalerao (1936) found it in India. Vazquez-Colet and Africa (1938, 1940) found that the fish, *Mugil dussumieri, Hemiramphus georgi, Ambassis buruensis, Arius manilensis, Clarias batrachus, Gerris kapas* and *Ophiocephalus striatus* served as second intermediate hosts to *H. yokogawai* in the Philippines. Kuntz and Chandler (1956) reported this species from the dog, fox, and osprey in Egypt.

Stellantchasmus falcatus was first described by Onji and Nishio (1924) from the small intestine of amidori (a bird), dogs and cats in Japan. They found the metacercariae of this species in the muscle of the fish, *Mugil* and *Liza.* The genus *Liza* has been placed in the synonymy of *Chelon* by Schultz *et al.* (1953). Witenberg (1929) found this trematode in dogs and cats in Palestine but called it *Diorchitrema pseudocirrata* n. g., n. sp. He, too, found the metacercariae in *Mugil.*

Africa and Garcia (1935) were the first to report this species from man. Vazquez-Colet and Africa (1938, 1939, and 1940) found the metacercariae in *Anabas testudinalis* and *Mugil dussumieri* in the Philippines. Alicata and Schattenburg (1938) found this worm in man in Hawaii and stated that *Diorchitrema pseudocirrata* was a synonym of *Stellantchasmus falcatus*. Glover and Alicata (1957) reported another infection in a Hawaiian. Other species assigned to the genus *Stellantchasmus, S. formosanus* Katsuta, 1931, which experimentally parasitized man, dogs, cats, and mice to which mullet had been fed and *S. amplicoecalis* Katsuta, 1932b, from dogs, cats and mice, were considered synonyms of *S. falcatus* by Chen (1951). Kuntz and Chandler (1956) reported *S. falcatus* as common in Egyptian cats.

MATERIAL AND METHODS

Infected *Stenomelania newcombi* and *Tarebia granifera* were isolated in finger bowls. Emerged cercariae were studied alive and as fixed and stained whole mounts. Cercariae were fixed without pressure by forcibly ejecting them into cold Bouin's or Heidenhain solution. Earlier larval stages and percentages of infection were obtained by crushing snails. Some of the mullet, *Mugil cephalus,* exposed to cercariae were collected near San Felipe, Baja California. No previous infections were found in any of them. Goldfish, *Carassius auratus* (L.), were purchased from aquarium shops. *Gambusia affinis, Kuhlia sandvicensis, Lebistes reticulata, Tilapia mossambica,* and *Clarias fuscus* were also exposed to cercariae. *Kuhlia sandvicensis* were collected in salt water (Kaneohe Bay) and gradually adapted to fresh water, and the others (except *Clarias fuscus,* Chinese catfish, kindly donated by the University of Hawaii Aquarium) in fresh water ponds or streams of Coconut Island and Oahu. Hatchery-raised chicks and laboratory-raised cats and rats were used as experimental definitive hosts. Larval and adult specimens were fixed in cold Bouin's or Heidenhain solution, stained with Mayer's paracarmine or Celestin blue-B and mounted in Permount or H–S–R Mounting Medium. Serial sections were stained with hematoxylin and triosin.

All measurements are in millimeters unless otherwise stated.

OBSERVATIONS AND DISCUSSION

The parasites described in this paper develop in one or both of the snails, *Stenomelania newcombi* and *Tarebia granifera* in Hawaii. In all of the taro patch ditches examined to date *T. granifera* far outnumbers *S. newcombi.* The percentages of infection vary with the size of the snails and the locality in which they are collected. With little seasonal change and few migratory birds, the percentages of infection probably do not change during the year to the degree found in some regions (Martin, 1955). A total of 419, 201 *S. newcombi* and 218 *T. granifera,* were collected on June 5, 1957, crushed and examined. Of the *S. newcombi,* 16.9% were negative, 38.6% harbored *Stellantchasmus falcatus,* 26.8% *Centrocestus formosanus,* 16.4% *Haplorchis yokogawai* and 1.5% a megalurous type of cercaria. No multiple infections were found. Of the *T. granifera,* 63.8% were negative, 21.6% had *S. falcatus,* 7.3% *H. taichui,* and 7.3% the megalurous cercaria. From these findings it appears that *Haplorchis taichui* will not develop in *Stenomelania newcombi* and *Tarebia granifera* will not serve as host to *Centrocestus formosanus* and *Haplorchis yokogawai. T. granifera* seems to be a more favorable host to the megalurous cercaria than *S. newcombi.* Both snails serve as hosts to *S. falcatus,* the most frequent infection. The 419 snails mentioned above were selected for size, the *S. newcombi* at least 2.5 cm in length and the *T. granifera* at least 1.5 cm. In both species there is a directly proportionate relation between the number of infections and the size of the snails. For example, 80 *T. granifera,* at least 10 but less than 15 mm long, were 82.5% negative, 8.75% with *S. falcatus* and 8.75% had *Haplorchis taichui.* Com-

parable results were obtained with the small *S. newcombi*. The small snails have a higher percentage of negative individuals. The variation in infection percentages in different localities will be the subject of another paper.

Centrocestus formosanus (Nishigori, 1924)
(Figs. 1–5)

Approximately 27% of the larger *Stenomelania newcombi* collected were infected with *Centrocestus formosanus*. In the mantle tissue near the gills slender, elongate sporocysts were found in some of the snails. When straightened, some of the sporocysts were 1 cm in length. Young sporocysts are solid but older ones have a core of cells at least partially separated from the wall (Fig. 1). Clusters of nuclei within the core form the rediae which migrate to and develop within the digestive gland of the snail. Young rediae are heavily nucleated throughout (Fig. 3) but in older rediae most of the nuclei are grouped into germ balls. The rediae bear spines and bristles at their anterior ends. Measurements of 24 fixed and stained rediae containing only immature cercariae are: length 0.29–0.45, av. 0.37; width 0.084–0.126, av. 0.10; and pharynx 0.011–0.016, av. 0.014 long and 0.011–0.019, av. 0.015 wide. Only 1 generation of rediae was observed.

The cercariae are biocellate with a double left ocellus and a single right. The anterior ¼th of the body is spined. There are 9 prominent spines immediately anterior to the mouth and a few fine bristles extend from the sides of the body. Light-brown granules are scattered throughout the body. Fourteen penetration glands have their cell bodies located between the pharynx and the excretory bladder. Their ducts pass anteriad to terminate in 4 bundles of 3–4–4–3 near the preoral spines. Cystogenous glands are laterally arranged between the pharynx level and the posterior end of the body. The oral sucker is well developed but the acetabulum is rudimentary. The digestive tract is incomplete consisting of a short prepharynx, a spherical to pyriform pharynx; a short esophagus and a rudimentary intestine. The genital primordium consists of a mass of cells immediately anterior to the excretory bladder. The excretory bladder is thick-walled and varies from a flattened sack to an X-shape. The main excretory tubes arise from the anterolateral portions of the bladder and proceed anteriorly in a somewhat contorted fashion to mid-body level where they divide into anterior and posterior branches. Each branch bears 2 pairs of flame cells. The flame cell pattern is expressed by the formula $2[(2+2)+(2+2)]=16$. The tail is slender and bears a weakly developed dorsoventral fin. The fin extends over approximately the posterior ¾ths of the tail although it does not extend forward ventrally quite to the level of the dorsal starting point. A few nuclei are clustered near each end of the tail. Measurements of 25 fixed and stained specimens are: body length 0.047–0.122, av. 0.088; body width 0.026–0.071; av. 0.05; oral sucker length 0.018–0.026, av. 0.023; oral sucker width 0.015–0.023, av. 0.018; prepharynx length 0.001–0.007, av. 0.004; pharynx length 0.005–0.009, av. 0.008; pharynx width 0.006–0.008, av. 0.008; tail length 0.062–0.098, av. 0.078; maximum tail width 0.011–0.015, av. 0.013.

The cercariae leave the snail and swim slowly in the water. If they come in contact with the gills of a suitable host they quickly shed their tails, penetrate and encyst. Fish with thick gills are more favorable hosts than those with slender gill filaments. *Mugil cephalus* and *Tilapia mossambica* have slender gill filaments and only extremely light natural infections have been found in the former, and the latter did

not become infected even after exposure to hundreds of cercariae. Experimental infections were produced by exposing goldfish (Fig. 3), *Gambusia affinis* (Baird and Girard), and *Xiphophorus helleri* Heckel. Natural infections occur in *Gambusia affinis* and *Xiphophorus helleri* from the taro patch ditches where the snails were collected. Forty *Gambusia* and 3 *Xiphophorus* from these ditches were examined. They varied from 1 to 3 inches in length. Only 3 of the *Gambusia* were negative. The remaining 37 had from 3 to 678 metacercariae per fish in the gills. They averaged 59.9 metacercariae per fish. The fish having 678 was only 2½ inches long. The 3 *Xiphophorus* contained from 250 to 500 metacercariae and averaged 411 per fish. The heavy natural infections probably have been accumulated over some period of time because a single experimental exposure to large numbers of cercariae may result in the death of the fish. The metacercarial cysts are oval with relatively thick walls. In fully developed metacercariae the eyespots break down but the pigment granules are still evident, the circumoral crown of usually 32 spines arranged in 2 rows and characteristic of the adult are developed, the digestive tract is complete and the ceca are filled with disc-shaped concretions, the excretory bladder becomes X- or I-shaped and is filled with globular concretions differing chemically from those of the intestinal ceca (the subject of another paper), and the reproductive organs are discernible.

Experimentally infected fish were fed to hatchery-raised chicks, white mice, white rats, and cats. Although all of these animals were positive, the rats and cats were more favorable hosts in that a greater percentage of the metacercariae fed became adult and more eggs were present in the uteri than in those developing especially in the chick. The adult *Centrocestus formosanus* were localized in the anterior portion of the small intestine. One rat fed several hundred metacercariae on May 28, 1956 was autopsied June 27, 1956 with the following results: 14 worms in the first 3 inches of the small intestine, 3 in the second, 3 in the third, 3 in the fourth, 1 in the fifth, 1 in the sixth; and the remainder of the intestine was negative. A cat fed 4 infected goldfish July 23, 1956 and examined August 6, 1956 had 148 worms in the first 6 inches of the small intestine, 157 in the second, 12 in the third; and the remainder of the intestine negative. Another cat fed 1813 metacercariae on April 3, 1957 and examined April 10, 1957 had 191 worms in the first 3 inches of the small intestine, 347 in the second, 113 in the third, 17 in the fourth, 5 in the fifth; and the rest of the intestine was negative.

A good description of the adult worm has been given by Chen (1942) who found rats and cats to be natural hosts in China. He indicated that man might become parasitized by eating partially cooked frogs. Natural hosts of *Centrocestus formosanus* in Hawaii include the night heron, *Nycticorax nycticorax,* rats and probably occasionally cats and dogs.

In the present description of the life cycle of *C. formosanus* certain findings are new and some vary from previous descriptions. The sporocyst stage has not been previously described. Chen (1948) showed only 4 flame cells in the cercaria but 16 were found by the present author. The latter number agrees with the flame cell count given by Yamaguti (1938) for the cercaria of *Centrocestus armatus* (Tanabe). Yamaguti showed only 4 penetration glands on each side of the body in *C. armatus* but this probably is incorrect. Chen (1948) reported a variation of 30-36 circumoral spines in the adult *C. formosanus*. In the present work, a variation of 31–34 circumoral spines was found in 24 worms. All but 5 of these had 32 spines.

Stenomelania newcombi Lea is a new first intermediate host. New second intermediate hosts are: *Kuhlia sandvicensis* (Steindachner), *Mugil cephalus* (Baird and Girard) and *Xiphophorus helleri* Heckel. This is the first report of *Centrocestus formosanus* in Hawaii, where it has been introduced probably from the Orient. Previously it has been reported from Formosa, Japan, China, and the Philippines.

Stellantchasmus falcatus Onji and Nishio, 1924
(Figs. 6–13)

Approximately 39% of the *Stenomelania newcombi* and 22% of the *Tarebia granifera* collected were infected with *Stellantchasmus falcatus*. Two generations of rediae developed in the digestive glands of these parasitized snails. Rediae vary from almost spherical to slender and elongate. The mouth opens into a small, nearly spherical pharynx which is connected to the saccular gut. In young rediae the gut may be ¾ths the body length but in older specimens it is proportionately much shorter. There is a birth pore near the anterior end. The redial wall contains yellow globules. Measurements of 24 rediae that contained only germ balls are: length 0.112–0.42, av. 0.234; width 0.056–0.112, av. 0.087; pharynx length 0.015–0.022, av. 0.018; pharynx width 0.015–0.022, av. 0.019.

The cercariae are ocellate and pleurolophocercous. The left eyespot is double and the right single. Brown granules are scattered through the body. The body cuticle is covered with spines arranged in a quincunx and decrease in size and number posteriorly. A few lateral bristles project from the body surface. The oral sucker is well developed but the acetabulum is rudimentary. Anterior to the mouth is a spined area consisting of 3 rows, with usually 5 elongate spines whose points project slightly into the mouth in the first row and 7 to 10 shorter spines in each of the other rows. The digestive tract includes a short prepharynx, small pharynx and rudimentary intestine. Fourteen penetration glands are arranged in 2 rows. The cell bodies of these glands extend from a short distance posterior to the pharynx to near the posterior end of the body. Their ducts pass anteriad and divide into bundles of 3–4–4–3 near the anterior end of the body. Cystogenous glands are laterally arranged from a little behind the eyespots to near the posterior end of the body. The thick-walled excretory bladder varies from the simple saccular to V-, Y-, or X-shaped. The main collecting tubes proceed anteriad from the anterolateral margins of the bladder to about the mid-body level where they divide into anterior and posterior branches each of which bears 3 groups of 3 flame cells each. The flame cell formula is $2[(3+3+3)+(3+3+3)]=36$. An exit tube from the bladder extends a short distance into the tail and divides into 2 branches which open laterally. The cuticle of the anterior 2/5ths of the tail is swollen and superficially annulated. A fin begins dorsally near the body then extends posteriorly around the tail tip and forward for about 2/5 ths of the tail length. Measurements of 25 fixed and stained cercariae are: body length 0.055–0.116, av. 0.091; maximum body width 0.034–0.064, av. 0.047; oral sucker length 0.018–0.023, av. 0.021; oral sucker width 0.016–0.02, av. 0.018; prepharynx length 0.003–0.011, av. 0.007; pharynx length 0.004–0.009, av. 0.007; pharynx width 0.006–0.009, av. 0.007; tail length 0.24–0.37, av. 0.32; maximum tail width 0.011–0.02, av. 0.017.

When the cercariae come in contact with the fish, *Mugil cephalus*, they quickly shed their tails and penetrate. Encystment occurs principally in the skeletal muscles. *Mugil cephalus* from the Gulf of California were exposed to cercariae and

extremely heavy infections resulted. The metacercarial cysts are oval to nearly spherical. Young metacercariae contain considerable pigment which, with the exception of granules from the eyespots, disappears as the metacercariae become older. Fully developed cysts are approximately 0.30 long and 0.28 wide. The cyst wall is composed of 2 layers, the outer granular and the inner clear. Changes from the cercarial stage include the completion of the digestive tract with the ceca filled with disc-shaped concretions, the development of the gonotyl (acetabulum of other authors) enclosed in a ventrogenital sac, the development of the testes, ovary and reproductive ducts, and the accumulation of concretions in the excretory bladder.

Natural infections have been found in *Mugil cephalus* collected in Kaneohe Bay. Every one of 25 fish examined was heavily parasitized, i.e. had several hundred cysts per fish in the muscles. Even the eye muscles may each contain several metacercariae. These fish probably became parasitized in fresh or possibly in slightly brackish water. In view of this infection rate it is surprising that more cases of human infection have not been reported. It is my opinion that if a survey were made of Hawaiians who eat raw mullet most of them would be found to harbor *S. falcatus*.

M. cephalus from the Gulf of California were exposed to large numbers of cercariae April 23–26, 1956 and were fed to 2 cats on May 4, 22, 29 and June 9 and 11, 1956. One cat was autopsied June 12, 1956 with the following results: the anterior 6 inches of the small intestine contained only a few worms, the second, third and fourth 6-inch lengths contained hundreds of worms each, the fifth and sixth had fewer and the remainder of the intestine was negative. Over fourteen hundred *S. falcatus* were recovered. The other cat examined July 25, 1956 presented much the same picture except that worms first appeared in the fourth 6 inches and were present in large numbers from there on through the eighth 6 inches. The remainder of the intestine was negative.

Measurements of 25 mounted specimens are: body length 0.257–0.387, av. 0.321; maximum body width 0.136–0.214, av. 0.176; oral sucker diameter 0.037–0.053, av. 0.045; prepharynx length 0–0.015, av. 0.004; pharynx length 0.025–0.04, av. 0.031; pharynx width 0.019–0.028, av. 0.024; esophagus length 0.031–0.081, av. 0.055; gonotyl length 0.028–0.038, av. 0.032; gonotyl width 0.028–0.035, av. 0.031; testes length 0.037–0.087, av. 0.058; testes width 0.034–0.068, av. 0.046; ovary length 0.028–0.043, av. 0.031; egg length 0.019–0.022, av. 0.02; and egg width 0.010–0.012, av. 0.011.

The body sizes of these worms are less than those given by other authors. They are nearest to those given by Katsuta (1931) for *S. formosanus*. These discrepancies probably are due to differences in age and/or fixation. The oldest of my specimens had been in the definitive host for 39 days. The ceca in these specimens usually reached to or near the anterior border of the testes whereas Chen (1951) described their extent as only to the lower level of the ovary. The ceca have relatively thick walls. The extent of the vitellaria from the ovarian level to near the posterior end of the body is in agreement with Chen (1951) but Witenberg (1929) showed them only in the testicular area. If this yolk distribution is constant in the Palestine material it may warrant raising Witenberg's specimen from synonymy to the status of a distinct species. Further study of the Palestine material is needed.

Other authors have called the muscular organ found in the ventro-genital sac, an

acetabulum. However, this cup-shaped structure has 2 opposed knobs that are covered with short, blunt spines and the lining of the cup bears scattered spines (Figs. 11–12). It seems to be comparable to the gonotyl of other species. Opposite the gonotyl and ventral to the terminations of the genital ducts there is a fleshy pad which may be a degenerate acetabulum.

Witenberg (1929) stated that the sperm expulsor had very thick walls in which spiral fibers were clearly seen. Chen (1951) stated that the expulsor wall was heavily chitinized. To my knowledge no specific tests for chitin in the expulsors of these worms have been reported. Spiral fibers were seen in the sperm expulsors of my specimens. They look like muscle fibers but no contraction was observed.

Price (1940) considered the genera *Stellantchasmus* and *Procerovum* to be closely related. However, the cercaria of *Procerovum* described by Hsu (1951) is quite different from that of *Stellantchasmus* and indicates that *Procerovum* is more closely related to the haplorchids than to *Stellantchasmus*.

Haplorchis taichui (Nishigori, 1924)
(Figs. 17–19, 21–23)

Approximately 7% of the *Tarebia granifera* collected were infected with *Haplorchis taichui*. Two generations of rediae develop in the digestive gland of these snails. The rediae are slender and elongate. The pharynx is small and spherical or nearly so and the gut is short. Measurements of 15 stained and mounted specimens are: length 0.264–.496, av. 0.408; maximum width 0.05–.084, av. 0.064; pharynx diam. 0.016–.022, av. 0.019.

The cercariae are biocellate with a single ocellus on the right side and a double one on the left. Intercellular brown granules are present from the mid oral-sucker-level to the posterior end of the body. The anterior part of the body is spined and there are a few bristles which extend laterally from the body surface. There is a preoral spined area consisting of usually 4 spines in the row nearest the mouth and 2 rows of 5 to 8 spines in each of the others. The mouth is surrounded by a cuticularized ring. There are 7 penetration glands on each side of the body. The cell bodies of these glands are lobed. They extend from a level just posterior to the pharynx to the posterior end of the body. Their ducts pass anteriad in 2 bundles which divide into a 3–4–4–3 arrangement at their terminations near the mouth. Cystogenous glands containing large granules are laterally arranged from the level of the pharynx to the posterior end of the body. The digestive tract consists of a well developed oral sucker, a short prepharynx, small pharynx and a rudimentary gut. A genital primordium is located immediately anterior to the excretory bladder. The excretory bladder is thick-walled and saccular. The flame cells are exceedingly difficult to observe because of body pigment, lack of contrast between them and surrounding cells and weakly beating cilia. Seven were seen on each side of the body but their capillary tubes could not be traced; 6 of these were in the anterior half of the body. An exit tube from the bladder extends a short distance into the tail where it divides into 2 branches which open laterally. The tail is elongate and slender and is set in a socket of the body. Its anterior third bears lateral fins, near the posterior ends of which a dorsoventral fin begins, extends posteriad around the tip of the tail and then anteriad ventrally to near the middle of the tail. Beneath the tail cuticle there is a cortex of longitudinal muscle fibers. Nuclei are distributed irregularly in the tail medulla.

Measurements of 25 stained and mounted cercariae are: body length 0.089–.124, av. 0.106; maximum body thickness (most specimens were viewed from the side) 0.031–.043, av. 0.035; oral sucker length 0.019–.025, av. 0.021; oral sucker width 0.017–0.02, av. 0.019; pharynx approximately 0.007 long and 0.006 wide; tail length 0.29–.35, av. 0.32; maximum tail thickness 0.016–.022, av. 0.017.

The cercariae leave the snail and swim about in the water. They are positively phototropic. Experimental infections were established in *Gambusia affinis, Mollienesia formosus* and in the goldfish, *Carassius auratus.* Natural infections have been found in *Gambusia affinis, Mollienesia formosus,* and rarely in *Mugil cephalus* collected in taro patch ditches at Kaneohe. The cercariae shed their tails, penetrate and encyst especially at the base of the tail and occasionally in the soft tissue around the mouth and pharynx. They sometimes encyst in the caudal fin of the goldfish.

The metacercarial size varies with age. Recently encysted metacercariae were approximately 0.14 long and 0.11 wide. Fully developed metacercariae were approximately 0.23 long and 0.16 wide. As the metacercaria ages it loses some of its body pigment but the eyespots are still visible, the body spines increase in size, the penetration glands disappear, many tubular, unicellular glands develop in the anterior part of the body especially around the oral sucker, the ceca develop and extend toward but do not reach the posterior end of the excretory bladder and become filled with disc-shaped concretions, the excretory bladder increases in size and becomes filled with globular concretions, the flame cells and excretory tubes become more prominent, the testis and ovary develop and the gonotyl with its armature becomes fully developed.

Adult worms were recovered from the small intestines of cats, some of which were fed experimentally infected *Gambusia* and others naturally infected *Gambusia.* They were located between the villi mainly in the first 24 inches of the small intestine. The major changes from the metacercarial stage include the development of vitellaria, sperm, and eggs. The gonotyl is pyriform with a cavity opening at its distal end. A semi-circle of 12 to 18 crescent-shaped bars is located on one side of the distal portion. On the inner surface of the gonotyl, adjacent to the bars, is a triangular patch of rods and spines (Fig. 23). The cecal walls are thick. Remnants of eyespots are present. The flame cell formula is $2[(2+2+2)+(2+2+2)]=24$.

Measurements of 20 stained and mounted, egg-bearing specimens are: body length 0.201–.353, av. 0.28; maximum body width 0.155–.263, av. 0.21; oral sucker diameter 0.025–.053, av. 0.037; prepharynx absent to very short; pharynx length 0.025–.037, av. 0.031; pharynx width 0.019–.028, av. 0.023; esophagus length 0.053–.087, av. 0.067; testis width 0.053–.074, av. 0.062; testis length 0.053–.087, av. 0.067; gonotyl length 0.034–.043, av. 0.039; gonotyl width 0.028–.037, av. 0.034; egg length 0.023–.026, av. 0.024; egg width 0.011–.014, av. 0.012.

Hsu (1951) stated that there are 2 flame cells on each side of the body in the cercaria of *H. taichui.* Those found by him are merely the ones most easily seen. As was stated previously, at least 7 flame cells are present on each side of the body of the cercaria. In the metacercariae and adults the flame cells and the excretory tubes are much more evident than in the cercariae. It is believed by the author that the excretory formula $2[(2+2+2)+(2+2+2)]=24$ applies to the cercaria as well as to the metacercaria and adult. The same grouping of flame cells in pairs but with only 4 pairs on each side of the body was found in the related species

Euhaplorchis californiensis by Martin (1950). Hsu (1951) also stated that the cercaria of *H. taichui* was among the smallest and the cercaria of *H. pumilio* the largest of the 5 heterophyid cercariae he described. However, if the cercaria of *H. taichui* (his figure 26) had been drawn to the same scale as the cercaria of *H. pumilio* (his figure 27) it would actually be larger than the latter.

This is the first report of *Haplorchis taichui* from Hawaii. Like *Stallantchasmus falcatus,* this species is widely distributed, having been reported previously from China, Egypt, Formosa, India, Israel, and the Philippines. Since it has been associated with human fatalities in the Philippines, it constitutes a health hazard in Hawaii.

Haplorchis yokogawai (Katsuta, 1932)
(Figs. 14–16, 20, 24)

Approximately 16% of the larger *Stenomelania newcombi* collected were infected with this species. Two generations of rediae develop in the digestive gland of the snail. The rediae are slender and elongate to saccular (Fig. 20). The pharynx is small and the gut short. Measurements of 10 rediae that contained only germ balls are: length 0.192–.465, av. 0.304; maximum width 0.046–.081, av. 0.067; pharynx length 0.019–.025, av. 0.022; pharynx width 0.0186–.025, av. 0.019.

The cercariae are similar to those of *H. taichui* in general morphology. However, in fixed specimens, on the average the body is shorter and less pigmented and the tail is longer than in the cercariae of *H. taichui.* In the first row of preoral spines *H. yokogawai* usually has 6 and *H. taichui* 4. Measurements of 20 stained and mounted specimens are: body length 0.068–.096, av. 0.082; maximum body thickness 0.037–.05, av. 0.041; oral sucker diam. 0.018–.023, av. 0.021; pharynx diam. approximately 0.009; tail length 0.310–.353, av. 0.331; maximum tail thickness 0.015–.021, av. 0.017.

A Chinese catfish, *Clarias fuscus,* was exposed to hundreds of cercariae for a period of 25 days. It was then cut into pieces and fed to 2 cats. The other catfish retained as a control was carefully examined and no metacercariae were found. One cat was autopsied 10 days after the experimental feeding and hundreds of *Haplorchis yokogawai* were found in the first half of the small intestine. The second cat was autopsied 14 days after the experimental feeding and the small intestine was cut into 6-inch segments and examined. Seven *H. yokogawai* were found in the first segment and over 100 in each of the second, third, fourth, fifth and sixth. The remainder of the intestine was negative.

The 10-day-old worms from experimentally infected cats contain only a few eggs each and are smaller than the 14-day-old worms. The cuticle is covered with spines which become minute at the posterior end of the body. Remnants of eyespots are present. The mouth is terminal or subterminal. The prepharynx usually is absent although a very short one was seen in extended specimens. The pharynx is elongate. The esophagus is well developed. The intestinal ceca terminate near the posterior margin of the testis. The testis is relatively large occupying much of the posterior fourth of the body. The oval ovary is immediately anterior to the testis and usually a little to the right of the mid-line. Near the ovary is a seminal receptacle of variable size. The vitelline follicles are scattered from the posterior margin of the gonotyl to near the posterior end of the body. The gonotyl is pyriform and 3-lobed with 2 sclerotized hooks as shown by Kuntz and Chandler (1956).

It usually is to the right of the mid-line. There is an oval seminal vesicle immediately posterior to the gut bifurcation. The eggs are elongate and operculate.

Measurements of 20 stained and mounted, 10- to 14-day-old specimens are: body length 0.27–.46, av. 0.36; body width 0.12–.21, av. 0.16; oral sucker length 0.031–.047, av. 0.037; oral sucker width 0.037–.047, av. 0.041; pharynx length 0.025–.034, av, 0.03; pharynx width 0.012–.022, av. 0.018; esophagus length 0.025–.05, av, 0.039; testis length 0.05–.093, av. 0.067; testis width 0.05–.078, av. 0.059; gonotyl approximately 0.042 long and 0.027 wide; ovary length 0.025–.05, av. 0.039; ovary width 0.019–0.047, av. 0.029; egg length 0.025–.031, av. 0.027; egg width 0.012–.016, av. 0.014.

One of 2 night herons, *Nycticorax nycticorax,* examined had many specimens of *H. yokogawai* in its small intestine.

Although Katsuta (1932a) stated that the mullet was the second intermediate host, no mullet were found to harbor this parasite in the present study. The Chinese catfish, *Clarias fuscus,* is found in some taro patch ditches and probably serves as a natural second intermediate host. Africa (1938) stated that *Haplorchis yokogawai* was the most frequently encountered haplorchid in man and the most frequently associated with cardiac failure. This species, therefore, constitutes a health hazard in Hawaii.

SUMMARY

The heterophyid trematodes: *Centrocestus formosanus* (Nishigori, 1924), *Stellantchasmus falcatus* Onji and Nishio, 1924, *Haplorchis taichui* (Nishigori, 1924) and *H. yokogawai* (Katsuta, 1932) have been found in Hawaii. *C. formosanus, H. yokogawai* and *S. falcatus* develop in the digestive gland of the fresh water snail, *Stenomelania newcombi* Lea collected in taro patch ditches of Oahu. *H. taichui* and *S. falcatus* develop in the digestive gland of the snail, *Tarebia granifera* Lam. collected in the same ditches. *C. formosanus* uses *Gambusia affinis* and *Xiphophorus helleri* as natural second intermediate hosts. Occasionally one to a few metacercariae were found in the mullet, *Mugil cephalus.* Adult *C. formosanus* have been found in the night heron, *Nycticorax nycticorax,* and in rats. Experimental infections were obtained in rats and cats. *Stellantchasmus falcatus* metacercariae were found in all mullet, *Mugil cephalus,* collected in Hawaii. Experimental infections were established in mullet collected in Baja California. Experimental infections of adult worms were established in cats. Natural infections of *Haplorchis taichui* were found in *Gambusia affinis, Mollienesia formosus* and, rarely, *Mugil cephalus.* Experimental infections were established also in the gold fish, *Carassius auratus.* Cats served as experimental definitive hosts. A natural definitive host is the night heron, *Nycticorax nycticorax. Haplorchis yokogawai* metacercariae were experimentally established in the Chinese catfish, *Clarias fuscus.* Cats served as experimental definitive hosts and the night heron, *N. nycticorax,* as a natural one.

All of these species of Heterophyidae constitute a health hazard in Hawaii.

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EXPLANATION OF PLATES AND FIGURES

(All drawings made with the aid of a camera lucida)

Abbreviations

b—excretory bladder; c—cystogenous gland; e—eyespot; ex—expulsor; f—flame cell; fi—fin; g—genital primordium, gonotyl in adults; i—intestine; m—metacercaria; o—oral sucker; ov—ovary; p—pharynx; pg—penetration gland; pr—prostate cells; s—seminal vesicle; t—testis; u—uterus; v—vitellaria; vs—ventro-genital sac.

PLATE I

FIGURE 1. Portion of a sporocyst of *Centrocestus formosanus.*
FIGURE 2. Cercaria of *C. formosanus.*
FIGURE 3. Portion of gill filament of *Carassius auratus* containing six metacercariae of *C. formosanus.*
FIGURE 4. Young redia of *C. formosanus.*

PLATE II

FIGURE 5. Older redia of *C. formosanus.*
FIGURE 6. Redia of *Stellantchasmus falcatus.*
FIGURE 7. Outline of cercarial body and some details of tail of *S. falcatus.*
FIGURE 8. Some details of cercarial body of *S. falcatus.*

PLATE III

FIGURE 9. Mature *S. falcatus,* ventral view.
FIGURE 10. Young adult of *S. falcatus,* ventral view.
FIGURE 11. Gonotyl of *S. falcatus,* ventral view.
FIGURE 12. Terminal genitalia of *S. falcatus.* Not all of the prostate cells are shown.
FIGURE 13. Metacercaria of *S. falcatus.*

PLATE IV

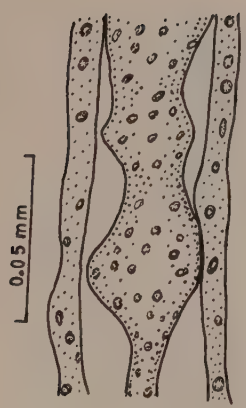
FIGURE 14. Outline of usual shape of Heidenhain-fixed cercaria of *Haplorchis yokogawai,* lateral view.
FIGURE 15. Preoral spine arrangement of *H. yokogawai* cercaria.

FIGURE 16. *H. yokogawai* cercaria, dorsal view.
FIGURE 17. Preoral spine arrangement of *Haplorchis taichui* cercaria.
FIGURE 18. *H. taichui* cercaria, ventral view of body, dorsal view of tail.
FIGURE 19. Outline of usual shape of Heidenhain-fixed cercaria of *H. taichui.*

PLATE V

FIGURE 20. Redia of *H. yokogawai.*
FIGURE 21. Redia of *H. taichui.*
FIGURE 22. Adult *H. taichui,* experimental infection in a cat.
FIGURE 23. Gonotyl of *H. taichui.*
FIGURE 24. Young adult *H. yokogawai,* ventral view.

PLATE I



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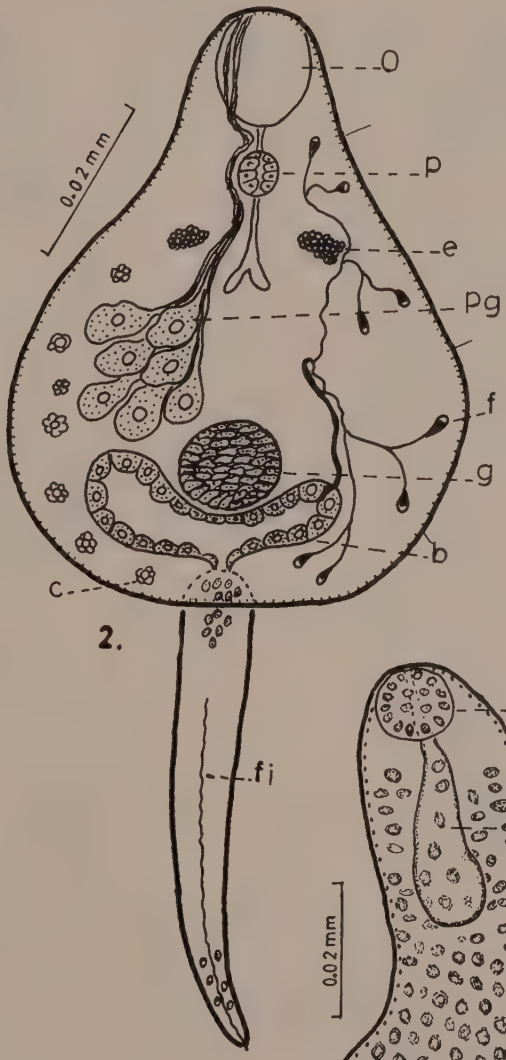

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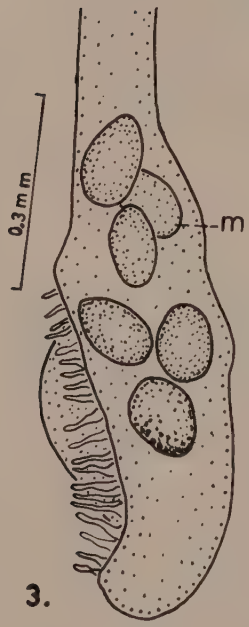

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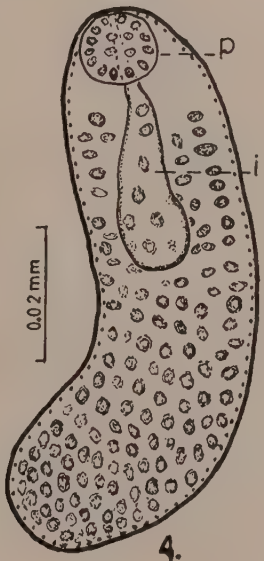

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PLATE II

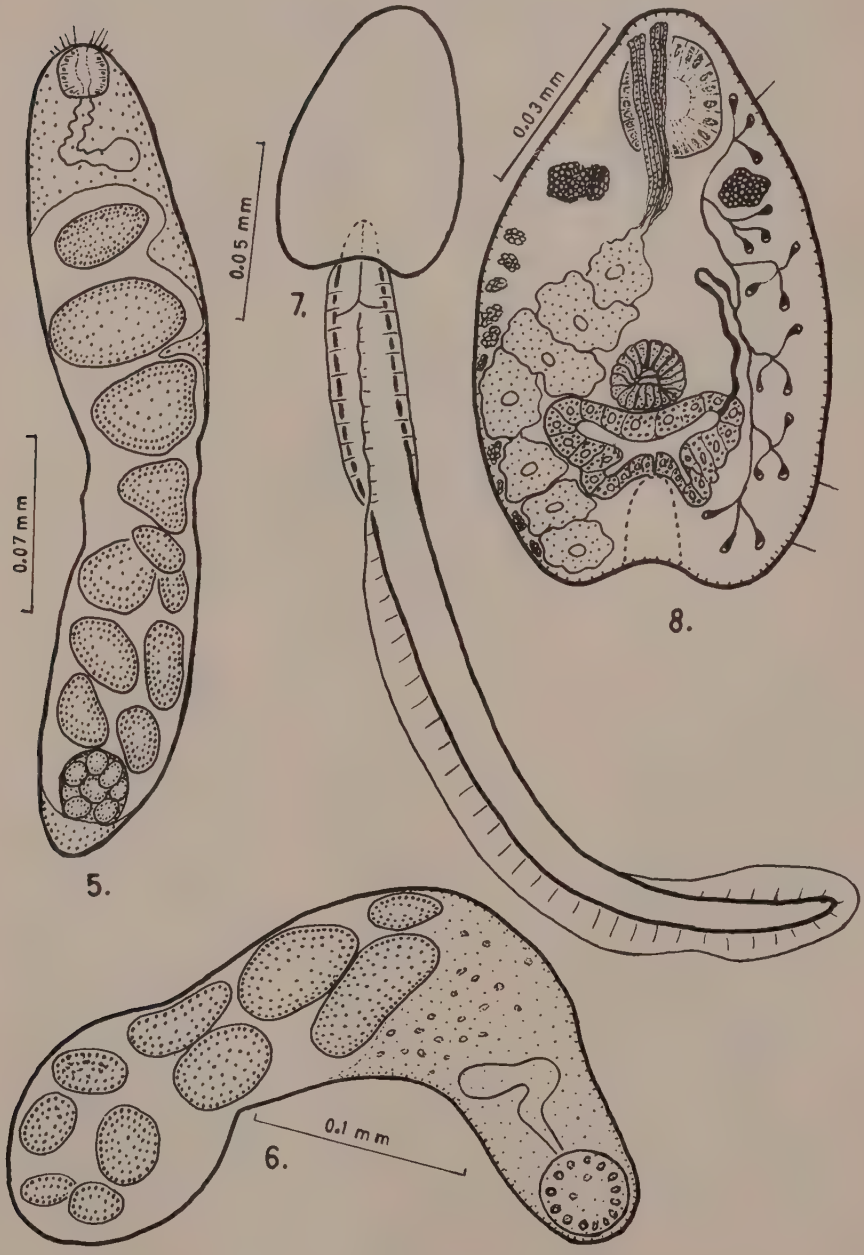


PLATE III

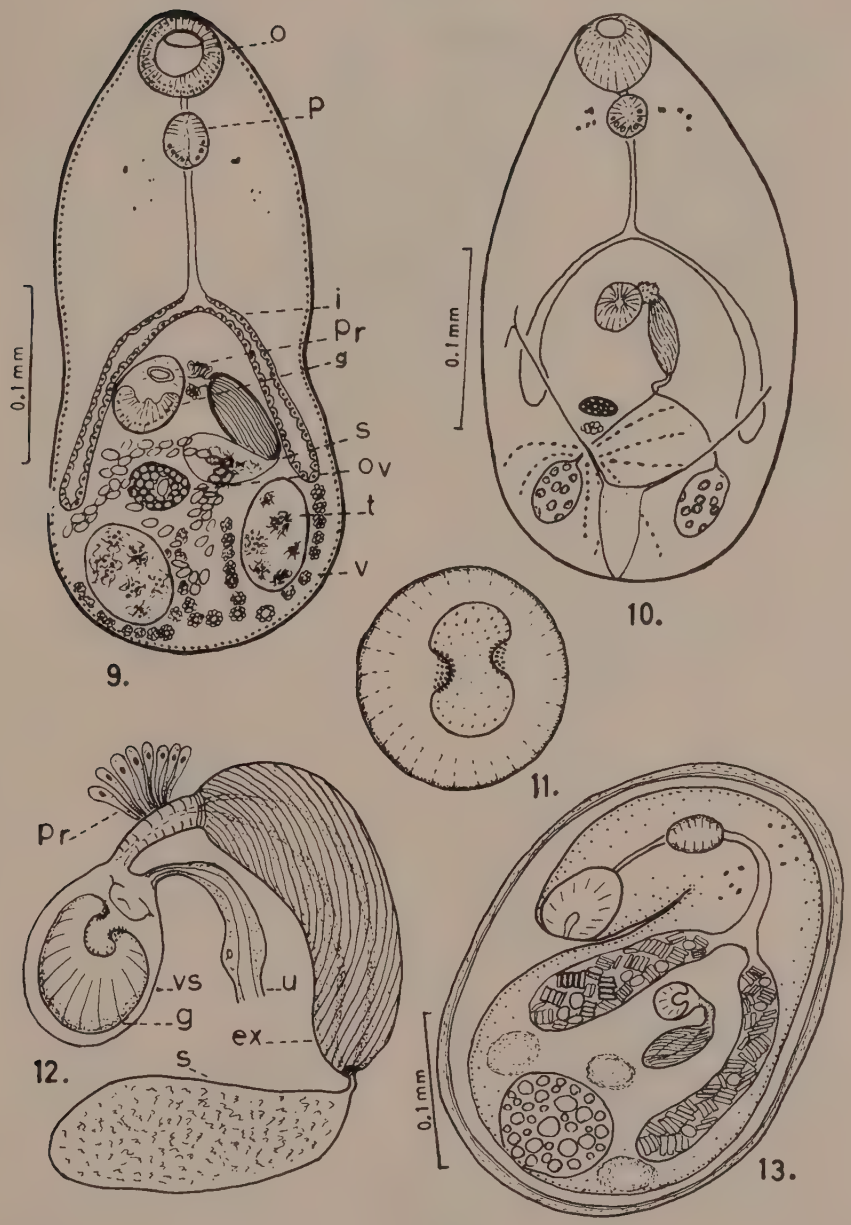


PLATE IV

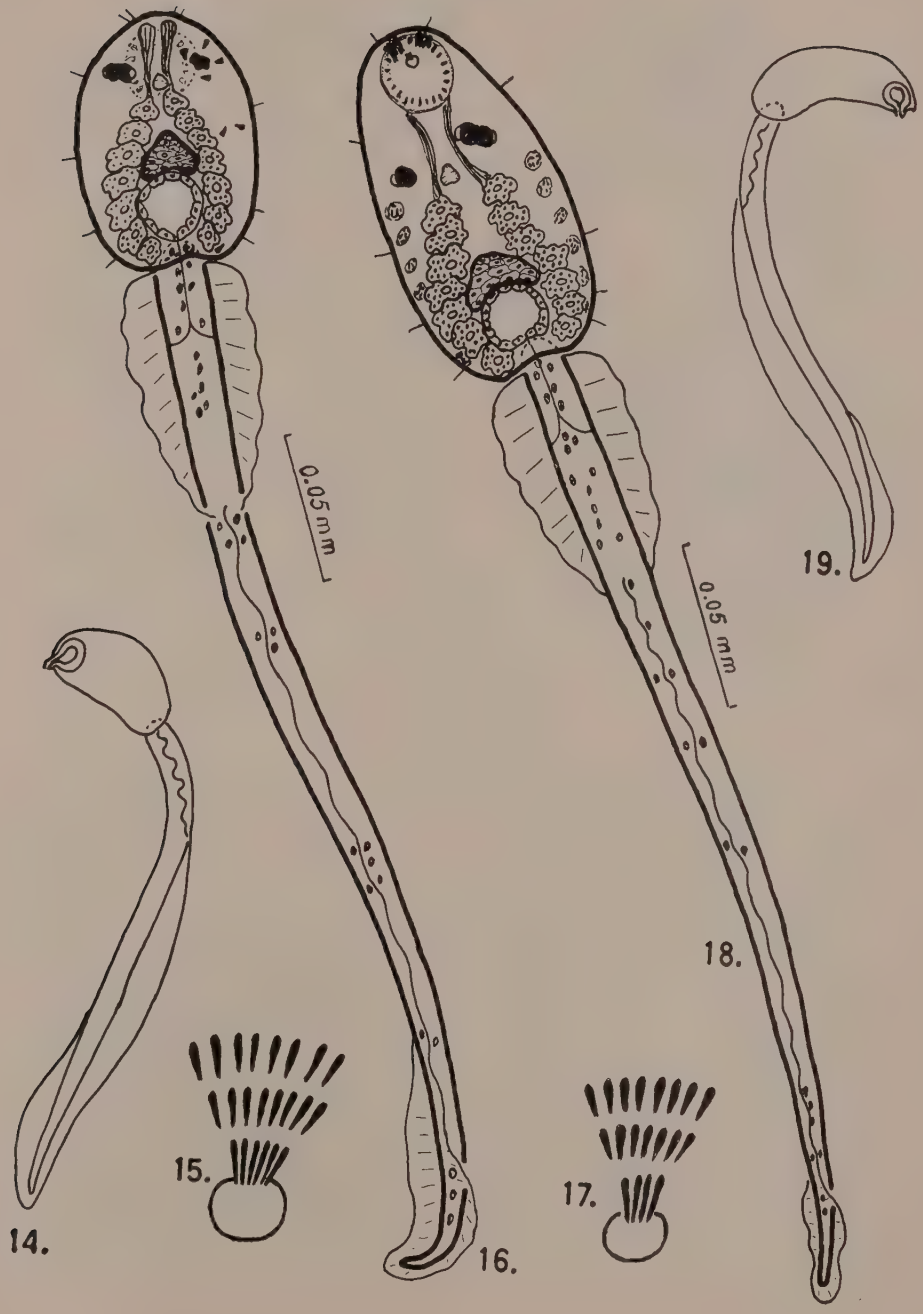
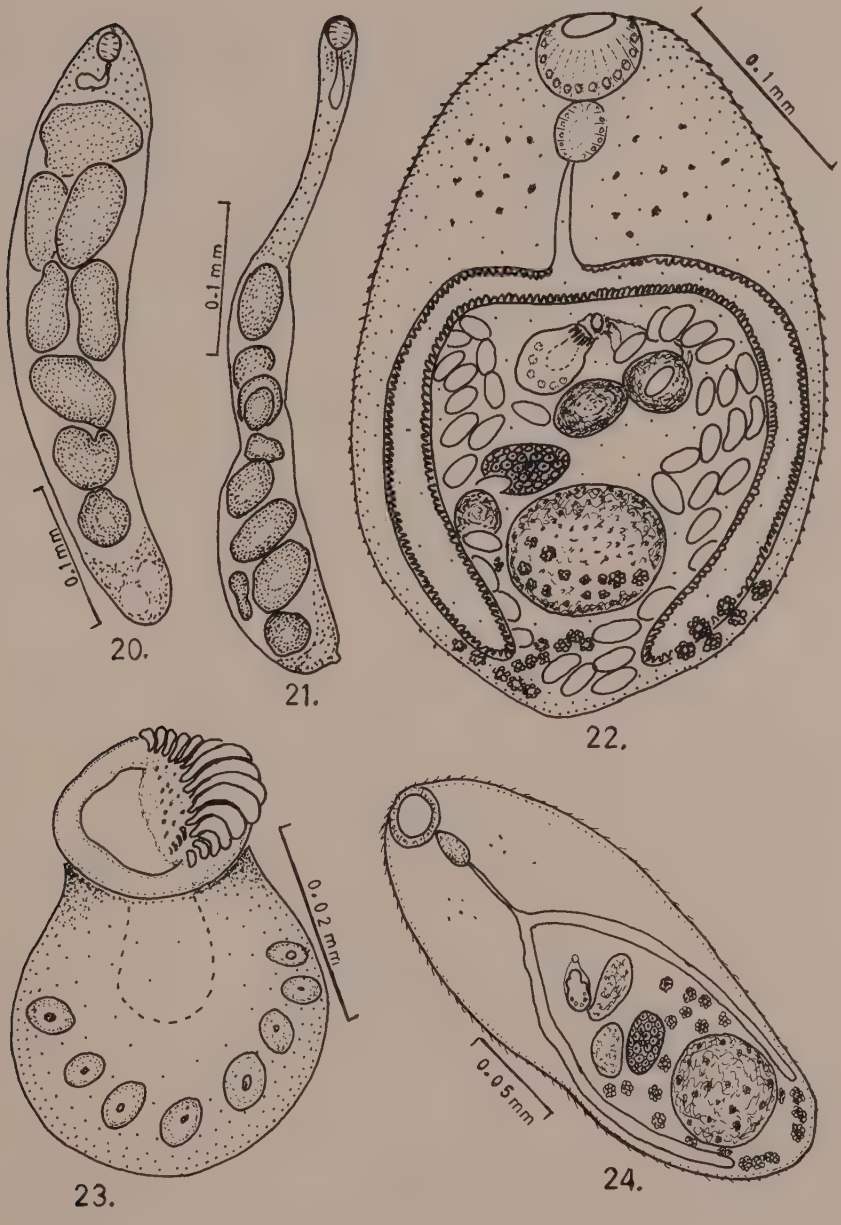


PLATE V



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The Accumulation of Y^{90} from an Equilibrium Mixture of Sr^{90}-Y^{90} by *Artemia salina* (L.)[1]

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ABSTRACT

Artemia salina (L.) was chosen as a representative organism in the second trophic level for studying one phase of the process of transfer of radionuclides through a marine food chain. Adult *Artemia* were cultured in filtered sea water which contained Sr^{90}-Y^{90}. They were not fed during the experiment which lasted 92 hours. They were removed at intervals, washed, and their radioactivity determined with an end-window counter. At 16 hours the concentration of radioactivity in the *Artemia* was about 40 times that of an equal weight of sea water. The radioactivity decayed at a rate which was similar to the decay rate of Y^{90}. It was estimated that only about 5 per cent of the activity remaining at secular equilibrium was due to Sr^{90}, the remainder being Y^{90}. This biological fractionation of Sr^{90}-Y^{90} may affect the transfer of these hazardous nuclides through food chains leading to human food. The fractionation of these nuclides by laboratory glassware is briefly discussed.

It has become increasingly apparent that in order to determine the biological hazards of radioisotopes from reactor wastes and from fallout we must increase our knowledge of both the rate of accumulation and the maximum concentration in the ecosystems involved. With such information we can more intelligently assess the importance of these isotopes in the biology of the individuals. It has been shown (Boroughs *et al.* 1956a, 1956b) that strontium and yttrium fed to fish are rapidly excreted. It has also been found that a relatively small amount is accumulated from the water. However, in all instances the amount retained seems to be dependent upon a number of factors, *e.g.*, species, age, food habits, and perhaps sex. It also appears that some organisms actually discriminate against one isotope in preference for another when both are present in the medium (Rice 1956). It is this effect which may reduce the concentration of isotopes such as Sr^{90} at various trophic levels.

Since the planktonic crustacea form a large percentage of the food at the lower trophic levels in the marine environment, they may serve as a means of transferring isotopes from the phytoplankton to the smaller carnivores. It was the purpose of the present work to determine the amount of Sr^{90} and Y^{90} accumulated by the brine shrimp, *Artemia salina* (Linnaeus), when these isotopes are present in sea water.

METHODS

All glassware used was freshly washed with concentrated H_2SO_4, detergent, and rinsed several times in distilled water. About 61.2 μc Sr^{90}-Y^{90} from Oak Ridge was diluted to 100 ml with sea water filtered through a glass fiber filter which retained particles of bacterial dimensions (0.1 microns). From this solution five 0.5-ml aliquots were pipetted onto aluminum planchettes and dried. Twenty-five adult *Artemia salina* were added to the sea water containing the Sr^{90}-Y^{90} mixture. Five brine shrimp were immediately removed from the solution, drained, and washed three times with distilled water. Preliminary experiments showed that very little radioactivity was lost by repetitive washing, and that distilled water and sea water washes were equivalent. The *Artemia* were then blotted with filter paper, arranged uniformly on an aluminum planchette, and dried under a heat lamp.

At intervals of 16, 40, 64, and 92 hours

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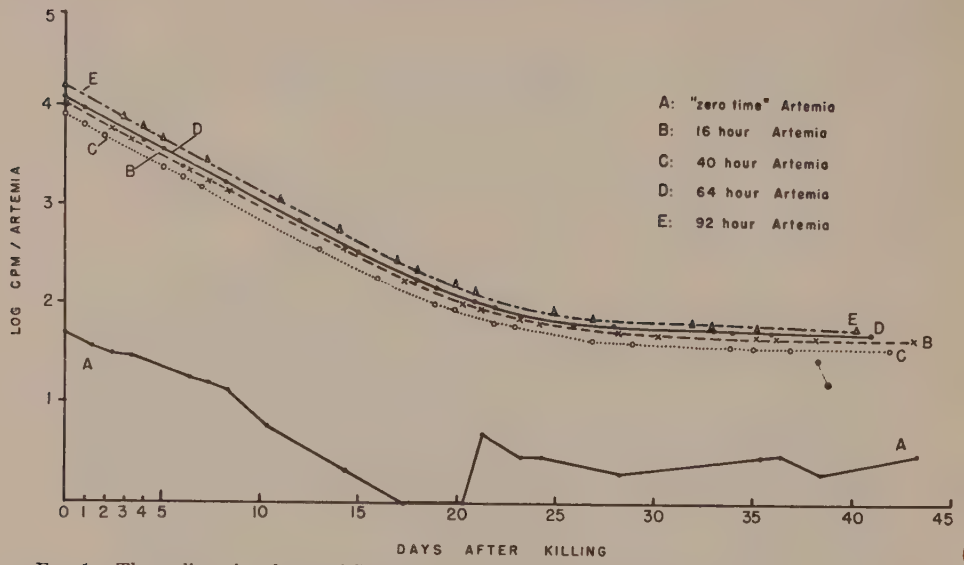

FIG. 1. The radioactive decay of Sr^{90}-Y^{90} in *Artemia* showing the accumulation of Y^{90} rather than of Sr^{90}.

later, five *Artemia* were removed and prepared as above. During this time the brine shrimp were not fed. When each group of brine shrimp was removed, 0.5-ml aliquots of the sea water were also pipetted onto aluminum planchettes and dried.

As each set of samples was prepared it was immediately counted on a commercial scaler using an end-window Geiger-Müller tube. All samples were counted daily in order to follow the radioactive decay, and appropriate corrections were made for coincidence when necessary. Owing to the high cpm, all samples were counted on the third shelf of a lead support, or approximately 4 cm from the Geiger-Müller tube.

Strontium^{90} from Oak Ridge is an equilibrium mixture of Sr^{90} and Y^{90}. Strontium^{90} has a half-life of about 28 years, and has a beta radiation with a maximum energy of 0.61 Mev. The daughter product of Sr^{90} decay is Y^{90} which has a half-life of about 2.5 days and has a beta radiation with a maximum energy of 2.18 Mev. At equilibrium the number of counts recorded is due to both the Sr^{90} and its daughter Y^{90}. If absorbers are used, or if there is a biological fractionation of the isotopes, the Geiger-Müller tube will count all particles entering it, but because more high energy particles enter the tube per disintegration than low energy particles, it will be more efficient for Y^{90}. A lapse of 10 half-lives of Y^{90} or about 25 days re-establishes the secular equilibrium of the isotopes. Thus it was necessary to count the samples at intervals for 25 days in order to be able to tell whether Sr^{90} or Y^{90} had been removed from the solution.

RESULTS AND DISCUSSION

Figure 1 is a semi-log plot of the radioactive decay of *Artemia* exposed to Sr^{90}-Y^{90} sea water for 0, 16, 40, 64, and 92 hours. It can be seen that when the brine shrimp were removed, the cpm/*Artemia* ranged from 8,200 to 12,000, but 43 days later it had dropped to 35–56 cpm/*Artemia*. From these curves several conclusions may be drawn. According to Curve A, there was no *immediate* binding of either isotope by the shrimp, but Curve B indicates that a large amount of radioactivity was taken up by the shrimp during the first 16 hours. The zero-time *Artemia* had about 55 cpm/*Artemia* when the samples were counted for the first time. These shrimp averaged 10.3 mg, so that 1 gram of *Artemia* would take up about 5,335 cpm. The 16-hour *Artemia*, on

the other hand, had about 10^6 cpm/gram. One gram of sea water had about 25,000 cpm. These numbers show that the *Artemia* which were merely dipped into the water did not absorb much of the radioactivity present, but that the 16-hour *Artemia* actually concentrated it about 40 fold (10^6/25,000).

The decay rate indicates that about half the initial radioactivity disappeared in 2.5–3.0 days, and this time is approximately equal to the half-life of Y^{90}. The high level of radioactivity, therefore, is due to the Y^{90} component, and the small residual radioactivity is due to the Sr^{90}.

The ratio of cpm/g *Artemia* to cpm/g sea water we call the concentration ratio. Table 1 shows the concentration ratios of the various samples at equilibrium. It can be seen that there is no significant change between the amount of Sr^{90} picked up after 16 hours and that picked up after 92 hours in the medium. On a weight basis one can therefore expect adult brine shrimp in a medium containing Sr^{90}-Y^{90} to accumulate about 13 per cent of the total available nuclides. At equilibrium, only half the disintegrations are due to Sr^{90}, but because of the difference in counting efficiency for the two different energy beta particles, it is thought that only about 5 per cent of the radioactivity at equilibrium is due to the Sr^{90}.

The concentration ratio at equilibrium is roughly the same regardless of the time the *Artemia* spent in the radioactive water (up to 92 hours). However, these results might be changed by longer periods of exposure, or by differences in age and sex of the animals.

It is apparent from the work of Chipman *et al.* (1955, 1956) that age is an important factor in determining the amount of Sr accumulated from solution. These workers exposed *Artemia salina* nauplii to Sr^{89}-Sr^{90}-Y^{90} in sea water for 24 hours. Table 2 shows their data calculated on the same basis as in Table 1. These data suggest that young *Artemia* accumulate more Sr from sea water than do adults. The higher concentration ratios may mean that *Artemia* which spend their entire lives in sea water containing Sr^{90}-Y^{90} will have more than the five per cent Sr^{90} we found with adults.

TABLE 1. *Concentration ratio of Sr^{90}-Y^{90} in adult* Artemia salina

Sample	cpm/g	Concentration ratio	Mean
Sea water	36,000		
Artemia—16 hrs	4,600	0.13	
Artemia—40 hrs	3,500	0.10	0.13
Artemia—64 hrs	4,900	0.14	
Artemia—92 hrs	5,600	0.16	

TABLE 2. *Concentration ratio of Sr^{89}-Sr^{90}-Y^{90} in* Artemia salina *nauplii*

Exposure time (hrs)	Sea water cpm	*Artemia* cpm	Concentration ratio
0.5	11	4.7	0.43
1	11	6.5	0.59
2	11	6.5	0.59
4	11	7.2	0.65
7	11	8.5	0.77
24	11	7.8	0.71

The amount of Sr^{90} taken up may also be increased if the Sr^{90} is present in phytoplankton which the *Artemia* eat.

Artemia, therefore, discriminate against the long-lived Sr^{90} in preference for the short-lived Y^{90}. It is possible that through such fractionation the more hazardous Sr^{90} may be lost by the time the higher trophic levels are reached. On the other hand, some organisms may concentrate strontium almost exclusively from an equilibrium mixture. Thus, Rice (1956) found that 90 or more per cent of the radioactivity in 10 species of algae was due to yttrium, but that *Carteria* accumulated only strontium. Such organisms as *Carteria*, therefore, may be a considerable source of contamination which can be transferred up the food chain. It is apparent that the loss of an isotope in one food cycle may promote the enrichment of this isotope in another cycle.

The radioactivity of the sea water samples was also followed from day to day. Figure 2 shows that at zero time the water had an activity of 15×10^3 cpm per 0.5 ml. Water samples which were removed 40, 64, and 92 hours later, however, had an activity of 12.5 to 13.5×10^3 cpm per 0.5 ml. At equilibrium the radioactivity in all the water samples had increased to $17–18.5 \times 10^3$ cpm per 0.5 ml. This was an increase in radioactivity of 20–60 per cent above that found when the samples were first counted.

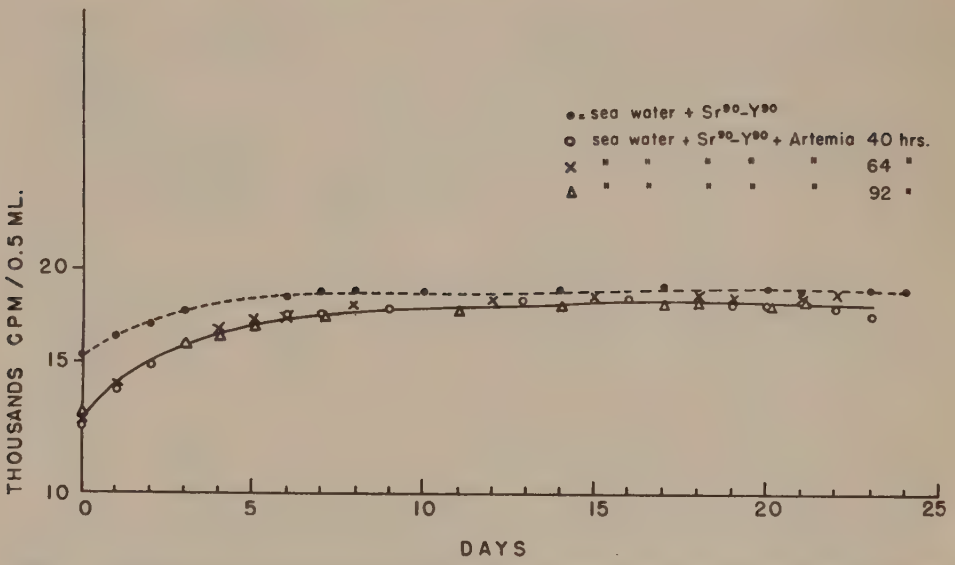

FIG. 2. The increase in radioactivity of the sea water medium, showing the build up of Y^{90} as a result of fractionation of the original Sr^{90}-Y^{90} mixture.

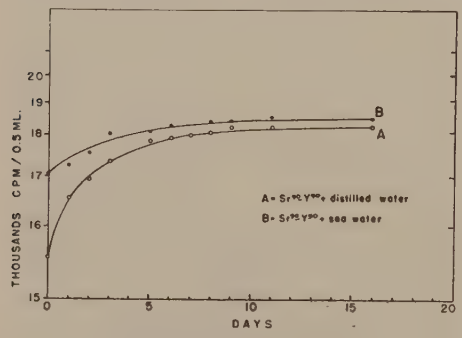

FIG. 3. The adsorption of Y^{90} by glassware from distilled water and sea water solutions of Sr^{90}-Y^{90}.

Of particular interest is the fact that the number of counts/minute for all the samples at equilibrium was approximately the same irrespective of the number of counts/minute when the samples were prepared (*cf.* 0^t dose and 92^h dose). This rise in activity implies that in all the samples some Y^{90} had been removed from the original equilibrium mixture.

It must be pointed out that using an equilibrium mixture of Sr^{90}-Y^{90} for such studies as these presents many problems, because it is difficult actually to assess the role of each isotope in the biological sense. This difficulty is due to the physical and chemical nature of the mother and daughter products.

Strontium is very likely ionic in sea water, but yttrium is probably at least partially colloidal. Strontium is bivalent and yttrium trivalent, and there is a significant difference in the size of their ionic radii. These differences mean that the biological binding sites for the two elements may be wholly different, but the sites may also overlap. If the sea water planchettes had been counted only at the time they were made, one would never suspect that there would be a significant change in the amount of radioactivity over a short time interval. Since Sr^{90} has a half-life of about 28 years, one would expect a constant count rate over a period of 30 days. However, when the isotope from Oak Ridge was pipetted into the sea water, Y^{90} was immediately lost, most likely by being adsorbed onto the glass. This means that each step in pipetting removed more yttrium from the mixture and upset the equilibrium. To test this hypothesis Sr^{90}-Y^{90} was added to flasks con-

taining sea water or distilled water. Figure 3 shows that more Y^{90} was adsorbed from distilled water than from sea water.

Thus it appears to us that in working with such isotopes which may have a tendency to become adsorbed onto surfaces, much care must be taken in the interpretation of data, since considerable variation may occur depending upon the time intervals concerned. Furthermore, it is apparent that it is very difficult to work with an equilibrium mixture of Sr^{90} and Y^{90} using ordinary glassware.

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THE EFFECT OF POISONOUS FISH UPON INTESTINAL PARASITES[1]

WALTER MARTIN[2] AND ALBERT H. BANNER[3]

Throughout the tropical Pacific there are numerous species of fish that are poisonous for human consumption in some localities but which are eaten with impunity elsewhere. These include such common fish as the moray eels (*Gymnothorax*), carangids (*Caranx*), snappers (*Lutjanus*) and surgeon fish (*Ctenochaetus*) (Halstead and Bunker, 1954). When poisonous members of these genera are eaten by Man they will cause reactions including nausea and vomiting, tingling or numbness in the mouth and lips, sensation reversal, (*i.e.*, hot seems cold), and in severe cases, impairment of motion, as in walking, and sometimes convulsions and death (Hiyama, 1943: 58)[4]. It has been established that the toxin is not the result of bacterial decomposition (*op. cit.*, pp. 13–15).

Hiyama (*op. cit.*, p. 58) also stated, "Some persons reported being cured of roundworms and feeling better than ever afterwards. This occurred with the *akadokutarumi* [*Lutjanus vaigiensis* (Quoy and Gaimard)] and the *dokuutsubo* [*Gymnothorax flavimarginatus* (Rüppell)]."

It has been established in experiments by the junior author and Howard Boroughs, working at the Hawaii Marine Laboratory, that whereas cats are violently affected by toxic fish, showing symptoms similar to those in human beings, rats eat poisonous fish with impunity; further, that the poison can be extracted from dried poisonous fish with 90 percent alcohol and evaporated on dry dog food without loss of toxicity, as shown by feeding tests on cats and mongooses (data as yet unpublished).

To discover the effects of poisonous fish upon intestinal helminths, two sets of experiments were performed, one with white rats, chosen because they could be fed many times a dosage of poisonous fish which, in terms of body weight, would be lethal if fed to cats or other sensitive animals. The other tests were with cats, which develop the typical intestinal symptoms and might, therefore, flush out narcotized parasites. The toxic fish were two specimens of *Lutjanus bohar* (Forskål)[5] from Palmyra Island, shown by previous experiments to be lethal when fed at the rate of 7.5 and ten percent body weight of the two test animals, respectively. The rats were fed raw fish and alcoholic extract evaporated upon dog food; the cats were fed raw fish.

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[1]Contribution No. 103, Hawaii Marine Laboratory, University of Hawaii.

[2]Department of Biology, University of Southern California, Los Angeles, Calif. These studies were aided by a contract between the Office of Naval Research, Department of the Navy and the University of Southern California, NR 165–252. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the naval service at large.

[3]The Department of Zoology and Entomology, University of Hawaii, Honolulu 14, Hawaii.

[4]The pagination in the references to Hiyama refer to the pages of Van Campen's translation—see Literature Cited.

[5]The authors wish to thank William A. Gosline, University of Hawaii for identification.

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TABLE I

The effect of poisonous fish upon intestinal helminths

Experiment	Test Animal	Material Fed	Amount Fed	No. of *C. formosanus* Fed	Duration of Experiment	No. of *C. formosanus* Recovered	No. of Cestodes Found	No. of Nematodes Found
I	1 Rat	a. Raw muscle *L. bohar* b. Extract on dogfood	All it could eat for one week	160	2 weeks	2	6	0
	1 Rat	a. Tuna b. Dogfood	Same	173	2 weeks	0	3	0
II	6 Rats	Extract of *L. bohar* on dogfood	All they could eat for duration of experiment	70 ea.	a. 8 days b. same	19 5	0 0	0 0
					c. 9 days d. same	10 2	0 0	0 0
					e. 12 days f. same	10 4	0 0	0 0
			Total for 6 test animals	420		50	0	0
	5 Rats	Dogfood	All they could eat for duration of experiment	70 ea.	a. 8 days b. same	1 3	0 0	0 0
					c. 9 days d. same	5 16	0 0	0 1larval
					e. 12 days	10	0	8 "
			Total for 5 control animals	350		35	0	9 "
III	1 Cat	Raw muscle *L. bohar*	5% body weight of cat (vomited much of test meal)	1813	1 week	673	Many	Some
	1 Cat	Raw muscle *Chaetodon* sp.	All it could eat	1816	1 week	542	0	0

The parasites experimentally introduced to the test animals were *Centrocestus formosanus* (Nishigori, 1924) (Trematoda: Heterophyidae) described from Formosa, and found commonly in the taro ponds of Hawaii. Metacercariae, released from melanid snails, were permitted to encyst in the laboratory in the gills of *Gambusia affinis* (Baird and Girard), *Xiphophorus helleri* Heckel, and the common goldfish, *Carassius auratus* (L.). The number of metacercariae in the fish gills was counted and then fed to test animals. This parasite has a rapid development, becoming ovigerous in a few days. Both rats and cats are favorable definitive hosts.

In the experiments, two days were allowed between the feeding of metacercariae and the poison fish. Control animals were fed non-toxic fish, either tuna, *Euthynnus yaito* (Kishinouye), or butterfly fish (*Chaetodon* sp.). The rats were given all of the poisonous fish or dog food with fish extract they could eat, and they ate many times the proportional amount that would cause death in sensitive animals. The rats used in experiment II were all litter mates and not fully grown. The test cat was fed five percent body weight of a fish that was established to be toxic at 7.5 percent body weight in mongooses. Although it ate the whole meal it vomited most of it within a few hours. In spite of losing a large portion of the toxic meal the cat developed the characteristic symptoms which included such acute paralysis that it could not stand. The results are presented in Table I.

SUMMARY AND CONCLUSIONS

Thirteen laboratory rats and two cats were given known numbers of metacercariae of *Centrocestus formosanus* (Nishigori) and divided into test and control lots. After two days, giving the metacercariae time to become established, the test animals were fed *Lutjanus bohar* (Forskål) of known toxicity either as raw flesh or as an alcoholic extract evaporated upon dog food. In none of the tests was the experimental infection reduced in the test animals. Moreover, the test animals also carried unidentified cestodes and nematodes at the conclusion of the experiment.

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Central Pacific Eels of the Genus *Uropterygius*, with Descriptions of Two New Species[1]

WILLIAM A. GOSLINE[2]

IN THE CENTRAL PACIFIC, at least, *Uropterygius* is, after the related *Gymnothorax*, the largest and most difficult genus of eels. Inadequate knowledge precludes any final revision at the present time either of the genus as a whole or of the species in the region under consideration. The individuals are, for the most part, among the smallest and most inconspicuous of morays, and some species doubtless remain undescribed. Nomenclatorially, the final allocation of the older names to the various forms must await examination of the types or of topotypical material. Where the specimens available do not aid in the solution of nomenclatorial problems, I have followed Schultz (1953: 140–159); to do otherwise would only be substituting one dubious name for another.[3]

The basic classification of the genus was laid down by Schultz (1943: 23–33; 1953: 140–159). The present paper could not have been written without this groundwork. It is, indeed, something of an addendum to Schultz's treatments of the genus, and full descriptions and synonymies have been omitted here. Its purpose is to clarify further the distinctions and relationships between Central Pacific species of *Uropterygius*, and to describe two new ones.

The genus, as here recognized, has the limits drawn for it by Schultz (1953: 99), i.e., *Scuticaria* is considered a synonym but *Anarchias* is excluded as a separate valid genus. Within the area under consideration *Uropterygius* may be sufficiently defined as containing those muraenid eels with no lateral-line pore near the posterior nostril, with sharp teeth in the jaws, and with the fins restricted to the tail region. (An alizarin-stained specimen of *U. knighti* shows that in this species, at least, the rayed portions of the dorsal and anal fins commence about one head length ahead of the tip of the tail.) The investigated members of the genus, except *U. xanthopterus*, seem to be differentiated from other muraenids in having a single open lateral-line pore above and before the gill opening. Other morays examined (*Muraena pardalis*, *Echidna polyzona*, *E. nebulosa*, various species of *Gymnothorax*, *Rabula fuscomaculata*, *Anarchias allardicei*, *A. cantonensis*, and *A. leucurus*) have two pores, one in front of the other, in this area (as does *Uropterygius xanthopterus*). The one exception is *Echidna zebra*, which does not have any pore.

The type of *Uropterygius* is *U. concolor* Rüppell from the Red Sea.

KEY TO THE CENTRAL PACIFIC SPECIES OF *Uropterygius*

1. Anus in front of or approximately under the middle of the length.....3

   Anus at least two-thirds of the way back along the length. Species reaching at least 3 feet in length.....2

2. Posterior nostril over front border of orbit; color plain brown.....**U. sealei**

   Posterior nostril over eye; color light with prominent round or oval dark blotches.....**U. tigrinus**

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[3] I believe that the same nomenclatorial policy could advantageously be followed for other Central Pacific fishes. That the scientific names applied to most of the fishes from this area can only be provisional may as well be admitted. It is merely a question of what provisional set of names to accept. The nomenclature of Schultz *et al.* should be altered, of course, as the various groups become revised on a world-wide basis. However, the adoption of such a policy should not be construed as a deterrent to further work on the zoological entities represented within the Central Pacific, or to the adoption of the nomenclatorial changes necessitated by such work.

3. Anus somewhat in front of middle of length; body without large, round or oval dark blotches.....5

   Anus under or slightly behind middle of length; body with large, round or oval dark blotches.....4

4. Vomerine teeth continuous with those on the premaxillary (Fig. 1*b*) and of approximately the same form; posterior nostril in a tube in specimens over 10 inches long; head without lengthwise bands.....**U. polyspilus**

   Vomerine teeth well separated from the premaxillary series, relatively small, conical; posterior nostril without a tube; head with lengthwise bands. **U. fijiensis**

5. Gill opening on middle or lower third of sides.....7

   Gill opening high, on upper third of sides. Teeth multiserial on the sides of jaws (Fig. 1*c*, *d*); eye in front of middle of wide gape.....6

6. Teeth in a broad band all along the sides of the upper jaw (Fig. 1*c*); head and chin spotted; maximum known length, 17 inches.....**U. supraforatus**

   Teeth in 3 or 4 rows along middle of the upper jaw, tending to become biserial posteriorly (Fig. 1*d*); head and chin plain; maximum known size, 10 inches .....**U. fuscoguttatus**

7. Teeth on the sides of the jaws in one or two rows; vomerine teeth uniserial or absent; maximum size about 10 inches.8

   Teeth on the sides of the jaws in three rows; vomerine teeth in two or three rows in front; maximum size 3 feet.... .....**U. marmoratus**

8. Teeth on the sides of the jaws in two rows; vomerine teeth present.....9

   Teeth on the sides of the jaws in a single row; no vomerine teeth (Fig. 1*e*). Color uniform brown.....**U. inornatus**

9. Markings of some sort always present.**10**

   Color uniform brown. Inner row of teeth on the lower jaw extending only about two-thirds of the way back along the outer row.....**U. concolor**

10. A single open lateral-line pore in front of and above the gill opening (for the approximate position of this pore, see Fig. 2); no scattered light punctulations on the head and body.....**11**

    Two open lateral-line pores in front of and above the gill opening, one before the other; numerous scattered light punctulations on head and body..... .....**U. xanthopterus**

11. Posterior nostril over or behind the middle of the eye, which lies over or behind the middle of the gape.....**12**

    Posterior nostril over the front of eye, which lies ahead of the middle of the gape.....**U. micropterus**

12. Tail more or less pointed, the terminal rays about two eye diameters in length; gape short, contained more than 3 times in the head length (to gill opening).... .....**U. makatei**

    Tail bluntly rounded, the rays at its tip short, less than an eye diameter in length; gape relatively wide, contained fewer than 3 times in the head length..... .....**U. knighti**

Uropterygius sealei Whitley
Table 1

TYPE LOCALITY: Society Islands. This species was originally described as *Scuticaria unicolor* by Seale; the specific name is said to be preoccupied.

MATERIAL EXAMINED: One specimen from the Honolulu aquarium.

The posterior position of the anus and the plain brown color together are said to be distinctive. The other two plain brown species of *Uropterygius* do not seem to reach more than a foot in length whereas our specimen of *U. sealei* is 3 feet long. The dentition of the available specimen is somewhat mangled. Apparently unknown outside of the Society and the Hawaiian Islands.

Uropterygius tigrinus (Lesson)
Table 1, Fig. 1*a*

TYPE LOCALITY: Society Islands. Several specimens examined from Johnston Island and the Hawaiian chain, 27 to 34 inches in length.

The posterior position of the anus, together with the round black markings on the sides, is distinctive. The great similarity between *U. tigrinus* and *U. polyspilus* will be discussed under the latter species. In tooth pattern, *U. tigrinus* and *U. sealei* are very similar and differ from all other members of the genus. The teeth of the inner maxillary row, those on the vomer, and the larger premaxillary teeth are all subequal in size. The inner maxillary and premaxillary teeth are distinctly separated by a gap which is not much smaller than that separating the vomerine teeth from those on the premaxillary. The mandibular teeth are biserial but the inner row stops short of the front of the eye.

Uropterygius polyspilus (Regan)
Table 1, Fig. 1*b*

TYPE LOCALITY: Tahiti. Two small specimens available from Johnston Island and one 17-inch individual from Honolulu, Hawaii.

The young of this species, up to at least 8 inches in length, may be recognized immediately by the abruptly light snout (yellow in life). However, the 17-inch specimen, which agrees with small specimens in other respects, lacks the light snout; its color pattern is an almost exact duplicate of that of *U. tigrinus*. Indeed the specimen was identified as *U. tigrinus* until the relatively forward position of the anus was noted. (Because of this similarity of color pattern the original description of *U. tigrinus* was checked to see that it did not really represent this species. Fortunately the description states that the anus is two-thirds of the way back along the body, which eliminates this possibility.) However, the dentition

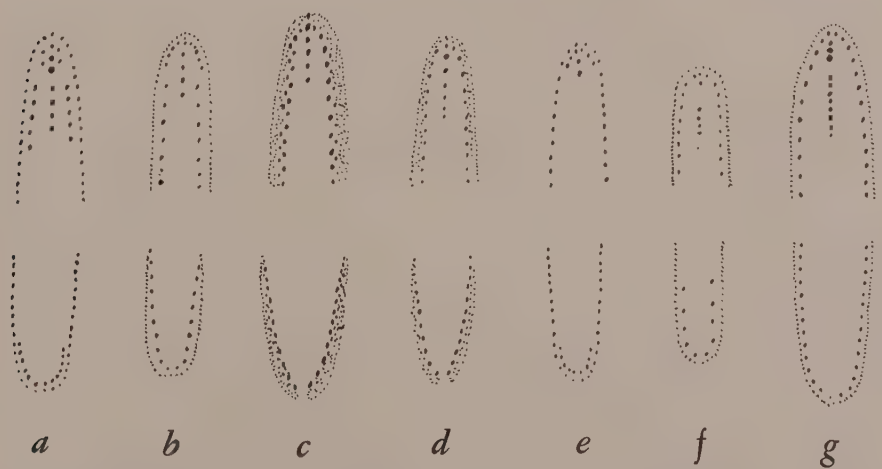

FIG. 1. Diagrammatic representation of dentition of *a*, *Uropterygius tigrinus*; *b*, *U. polyspilus*; *c*, *U. supraforatus*; *d*, *U. fuscoguttatus*; *e*, *U. inornatus*; *f*, *U. makatei*; *g*, *U. knighti*. Each dot represents a tooth; upper jaw above, lower jaw below.

TABLE 1
TOOTH COUNTS IN CERTAIN SPECIES OF *Uropterygius*

SPECIES	LOCALITY	LENGTH OF SPECIMEN IN INCHES	TEETH ON ONE SIDE			
			On outer row above[1]	On inner row above[1]	On outer row below	On inner row below
U. sealei	Oahu	36	10	10	15	5
U. tigrinus	Johnston	27	18	8	17	6
U. polyspilus	Oahu	17	35	7	30	8
U. xanthopterus	Line Is.	9.5	29	10	23	6
U. micropterus	Gilberts	9.5	43	15	40	11
U. knighti	Tuamotus	7	29	12	38	8

[1] These counts include both premaxillary and maxillary teeth within the rows indicated.

of the two species is entirely different. In the lower jaw of *U. polyspilus* the inner row of teeth extends about as far back as the outer, and the inner rows on both jaws as well as some of the teeth on the premaxillary and vomer are definitely enlarged and fanglike.

Uropterygius fijiensis Fowler and Bean

TYPE LOCALITY: Fiji. The type and only known specimen has been briefly redescribed by Schultz (1943: 24, 26, fig. 3f).

Judging from the descriptions the salient features of this species are: anus slightly behind middle of length; eye forward of middle of the moderate gape; rear nostril over front border of pupil; color pattern of five or six irregular rows of brown spots, more or less joined, and about eight or nine somewhat broken lengthwise bands on the sides of the head. The bands on the head would appear to be unique among Central Pacific species of *Uropterygius*.

The color pattern is somewhat reminiscent of *U. polyspilus* and *U. tigrinus* but the tooth pattern would seem to place it in an entirely different portion of the genus.

Uropterygius supraforatus (Regan)
Fig. 1*c*

TYPE LOCALITY: Savaii, Western Samoa.

SYNONYM: *U. dentatus* Schultz, with Johnston Island as type locality. Four specimens, 7 to 16 inches long, examined from the Gilberts, Johnston Island, and Honolulu.

The similarity between *U. supraforatus*, *U. dentatus*, and *U. fuscoguttatus* has been previously noted (Gosline, 1955: 155). There, in the absence of adequate comparative material, *U. supraforatus* and *U. dentatus* were both provisionally recognized. Subsequent examination of two Gilbertese specimens failed to show any basis for separating the two, which are herewith synonymized.

Schultz (1953) allocates specimens to both *U. supraforatus* and *U. dentatus*. His differentiation of the two species is based on color differences and on a gap between the vomerine and premaxillary teeth in *U. dentatus*. As to this gap, I find it no greater in our Johnston Island specimen topotypic for *U. dentatus* than for the Gilbertese specimens that appear to be typical of *U. supraforatus*. In color, the most significant difference between the Johnston and Hawaiian specimens at hand and those from the Gilberts is the heavier spotting on the former; but these specimens are also larger, and other species of *Uropterygius* also become more heavily pigmented with increasing size.

Uropterygius fuscoguttatus Schultz
Fig. 1*d*

TYPE LOCALITY: Bikini.

MATERIAL EXAMINED: Numerous specimens from Hawaii and Johnston Island.

About the only differences between this species and *U. supraforatus* seem to be those mentioned in the key. In the presence of

needlelike vomerine teeth far forward, though not in other characters, these two species somewhat resemble *U. sealei*, *U. tigrinus*, and *U. polyspilus*.

In the Hawaiian Islands this species occurs commonly in about 25 to 75 feet of water, but it was taken at a much shallower depth at Johnston Island, probably because of the very different reef conditions there.

Uropterygius marmoratus (Lacepède)

TYPE LOCALITY: New Britain.

SYNONYM?: *Ichthyophis pantherinus* Lesson. No specimens seen.

Lacepède's original description, based on Commerson, is of a marbled brown and white *Uropterygius*, a meter in length, with needlelike teeth. Lacepède's name has been applied to various members of the genus. Here, it is identified with the species called *Gymnomuraena marmorata* by Weber and de Beaufort (1916: 397, figs. 193, 194) and presumably represented by the larger specimens listed under *Uropterygius marmoratus* by Schultz (1943: 33; 1953: 155). The following combination of characters would seem to be distinctive for this species: large size, anus in front of middle of length, many rowed teeth, and gill openings about halfway up the sides. It would appear to be most similar to *U. supraforatus*.

In recent years Schultz (1943, 1953) has apparently confused two species in his accounts of *U. marmoratus* under the impression (mistaken, I believe) that the teeth and tooth rows increase with age. I can find no support for this supposition in available material of any species of *Uropterygius*.

Uropterygius inornatus new species
Table 2, Figs. 1*e*, 2*a*

HOLOTYPE: U.S.N.M. 175007, 187 mm. total length, off Waikiki reef, Oahu, in 25–35 ft. of water, Dec. 31, 1952, Gosline, Brock, Randall, *et al.*

PARATYPES: University of Hawaii No. 1686, 2 specimens, 108 and 129 mm., with the same data as the holotype; U.H. No. 1788, 1 specimen, 107 mm., cove 1 mi. north of Kailua, Hawaii, in 12–30 ft. of water, June 19, 1953, Brock, Gosline, *et al.*; U.H. No. 2362, 2 specimens, 117 and 189 mm., ½ mi. off Hawaiian Village, Waikiki, in 25–30 ft. of water, Sept. 7, 1951, Herald, Harry, Brock, Gosline, *et al.*

A very slender eel, heaviest just behind the head, and distinctly smaller near the tail (see Table 2). Caudal fin rays extending at least one eye diameter beyond the tip of the last vertebra.

The posterior nostril has no raised rim and opens above the middle of the eye; there is no lateral-line pore near it. The eye lies nearer the corner of the mouth than to the tip of snout, but the gape extends at least an eye diameter behind the eye. The usual lateral-line pores are present on the head, but on the jaws there are only 5 (sometimes 4 or 3) pores on each side instead of the usual 6. A single open lateral-line pore is present in front of and above the gill opening, as is normal for *Uropterygius*. The gill opening lies slightly above the midline of the sides.

The teeth are uniserial on the sides of the mouth. The only needlelike teeth are a few at the front of each jaw. There are no vomerine teeth in any of the six specimens.

The color is a plain, uniform brown though the tip of the tail and the chin are a lighter brown.

The uniserial teeth on the sides of the jaws and the absence of vomerine teeth in this species seem to be unique for *Uropterygius*. The closest relative of *U. inornatus* appears to be *U. concolor* Rüppell. (Certain counts and measurements of the only available specimen of the latter species, from Onotoa in the Gilbert Islands, are given in Table 2 for comparison.) *U. concolor* exactly duplicates *U. inornatus* in color, but appears to be a stockier, longer-headed eel with vomerine teeth and with a second, inner row of larger teeth running at least two-thirds of the way back in each jaw.

The name is derived from the Latin word *inornatus*, meaning undecorated.

Uropterygius concolor Rüppell

Table 2

TYPE LOCALITY: Red Sea. One specimen from the Gilberts examined, slightly less than 5 inches long.

This is one of three plain brown species of *Uropterygius* known from the Central Pacific. From *U. sealei* it differs in the more anterior position of the anus and in having the small, conical, vomerine teeth widely separated from the fanglike teeth of the premaxillary. *U. inornatus* does not have any vomerine teeth at all. Dental pattern in *U. concolor* is about as in Figure 1*f*; tooth counts and measurements for the available specimen are given in Table 2.

Uropterygius xanthopterus Bleeker

Table 1

TYPE LOCALITY: East Indies. Numerous specimens available from the Line, Tuamotu, and Marshall Islands.

Uropterygius xanthopterus is unique among available forms of the genus in having the usual two muraenid, but not *Uropterygius*, lateral-line pores above and ahead of the gill opening. The light speckling on a dark ground is equally distinctive. The tooth pattern is about as in Figure 1*f*.

Uropterygius micropterus (Bleeker)

Table 1

TYPE LOCALITY: East Indies. Schultz (1953: 156) considers *U. tinkhami* Fowler, type locality, Carolines, as a synonym of this species.

MATERIAL EXAMINED: One specimen, 9 inches long, from the Gilberts.

Judging from the specimen available, the forward position of the nostril and eye noted in the key are better distinguishing characters for this species than the color pattern; the vomerine teeth are biserial in front as shown in Schultz's figures and not uniserial as stated in his description (1953: 156). Except for the biserial vomerine teeth, the dentition is about as in Figure 1*g*.

TABLE 2

CERTAIN COUNTS AND MEASUREMENTS IN THREE SPECIES OF *Uropterygius*

Measurements, aside from total length and height of gill opening, expressed as thousandths of the total length

	U. inornatus		*U. concolor*	*U. makatei* (Holotype)
	Holotype	Five paratypes		
Total length (mm.)	187	107–189	123	230
Greatest depth	46	33–38	53	63
Depth at anus	34	30–37	47	50
Depth one head length before tail	24	20–28	35	40
Distance from tip of snout to anus	448	428–463	448	438
Head length, measured to gill opening	88	77–88	119	147
Tip of snout to corner of mouth	33	28–33	42	37
Snout length	20	16–22	20	19
Eye diameter	7	5–7	11	7
Distance from eye to corner of mouth	13	7–14	13	20
Length of middle caudal rays	9	6–9	7	13
Height of gill openings, in per cent distance up sides	50	58–67	58	20
Tooth counts				
Outer row above, on one side[1]	12	16–19	27	23
Inner row above, on one side[1]	2	0–4	8	9
Outer row below, on one side	13	14–20	28	25
Inner row below, on one side	3	2–5	4	5
Median teeth on premaxillary	3	2–3	3	3
Median teeth on vomer	0	0	7	4

[1]These counts include both premaxillary and maxillary teeth within the rows indicated.

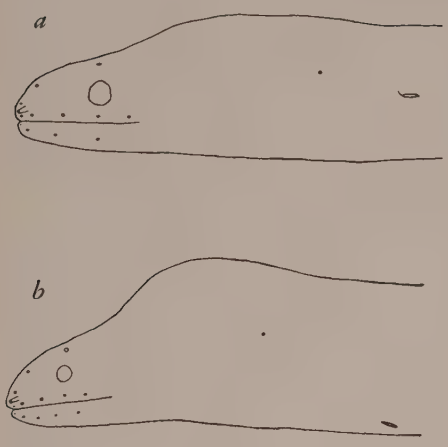

FIG. 2. Heads of holotypes: *a*, *Uropterygius inornatus*; *b*, *Uropterygius makatei*.

Uropterygius makatei new species

Table 2, Figs. 1*f*, 2*b*

HOLOTYPE: (Only known specimen.) U.S. N.M. 175008, 230 mm. in total length, taken just west of the phosphate dock, Makatea, northern Tuamotus, over reef flat and in surge channel, Mar. 15, 1956, by John E. Randall.

A short-jawed, heavy-headed, stocky *Uropterygius*. The tail is bluntly pointed with the terminal rays well developed for the genus.

The posterior nostril has a rudimentary rim and opens above the middle of the eye; there is no lateral-line pore near it. The small eye lies over the middle of the gape which is very short, contained almost four times in the head length. The usual lateral-line pores of the genus are present. The gill opening is very low, almost ventrally directed.

The teeth in the inner maxillary, premaxillary, and mandibular rows are moderately enlarged and fanglike. The vomerine teeth are smaller and essentially similar in size to those on the outer rows of the jaws.

The ground color is light with brown reticulations darkest on the snout and back, present on the mandible and lower surface of the body posteriorly, and almost completely lacking on the belly, which is plain gray. The anterior nostril and the head pores are of the same color as the surrounding areas, but the posterior nostril has a rather narrow white rim.

Uropterygius makatei seems to have shorter jaws in relation to the head length than any of the others taken up here. It also has the gill openings lower on the sides. This latter character places it with *U. knighti* in the key, but it has more of the look and coloration of *U. micropterus*. This last species has far longer jaws and more numerous teeth than *U. makatei*, however. In tooth pattern, the new species belongs with *U. concolor* and *U. xanthopterus*.

Named *makatei* for the island at which it was taken.

Uropterygius knighti (Jordan and Starks)

Table 1, Fig. 1*g*

TYPE LOCALITY: Samoa.

SYNONYM: *Uropterygius reidi* Schultz with type locality, Tau Island, Samoan group. Two specimens from the Tuamotus and one from Wake Island seem typical of the species; innumerable specimens from Hawaii, the Tuamotus, and Wake are provisionally identified with it.

The last section of Schultz's key (1953: 143, section 22b) seems to require comment. It leads to two species: *U. necturus* (Jordan and Gilbert) from the Gulf of California, and *U. knighti* (Jordan and Starks). Now according to Jordan and Evermann (1896: 404) *U. necturus* has "a pore situated just above the posterior nostril." If this is so, *U. necturus* is not a *Uropterygius* at all but an *Anarchias*. As for *U. knighti*, it is keyed by Schultz as having the "lower jaw notably barred or reticulated with brown"; however, Jordan and Starks' figure (in Jordan and Seale, 1906: 205, fig. 10) shows the whole lower jaw unpigmented and their description states that the mandible is white below. If this is true, there is little left to dis-

tinguish *U. knighti* from *U. reidi*, and I believe the two are synonyms.

At present I am also allocating to *U. knighti* a whole series of more or less dark-chinned specimens. It is presumably this type of fish that Jordan and Evermann (1905: 111) and innumerable others have been calling *U. marmoratus* and which is probably represented by the small specimens in Schultz's (1943: 33; 1953: 155) accounts of the same species. The reasons for provisionally combining the dark-chinned and light-chinned forms (cf. Schultz, 1953: 159) are: (1) that I can find no valid morphological differences between them, (2) that available collections (from Makatea, Wake) that contain the light variety also contain the dark one, and (3) that many individuals are partially intermediate between the two.

That the dark form is not the young of *U. marmoratus* can be more convincingly demonstarted: of the hundreds of Hawaiian specimens many are ripe adults but the largest is just over 13 inches long; in specimens from 3 to 13 inches there is no evidence for additional tooth rows with increasing size.

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Structure and Reproduction of *Cottoniella hawaiiensis* n. sp. (Rhodophyta)[1]

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M. RUTH WAINWRIGHT[2]

WHILE SORTING over fine red algae washed onto the beach at Kailua, Oahu, Hawaiian Islands, the junior author discovered an alga which appears to be representative of a previously undescribed specific taxon to be placed in the genus *Cottoniella* for reason of its obvious similarities to *Cottoniella arcuata* Boergesen, the type of that genus. Accordingly, this newly found material is described here as:

Cottoniella hawaiiensis n. sp.

Figs. 1–9

Thalli 2 cm. longi, ramis sterilibus polystichis ad 130 μ diametro et a 4 cellulis pericentralibus cinctis, cellula apicale 10–12 μ diametro in serie segmentis discoideis transversalibus partita, segmentis deinde 4 cellulis pericentralibus ferrentibus, ramis endogenis a apicibus cellulis centralibus productis et eis stichidiis tetrasporangialibus vel ramis vegetativalibus a ramo parenti simulantibus formantibus, ramis haplostichis determinatis ex angula ventrali anteriori cellulae lateralarum pericentralarum ferrentibus, stichidiis tetrasporangialibus jugum sporangiarum tetrahedro-partitarum ferrentibus, loculis sporangialibus ad 74 μ longa et ad 60 μ diametro.

The specimens were pale pink and, when disengaged from the other polysiphonous algae in which they were floating entangled, could be separated into lax unilateral branch systems up to about 2 centimeters tall (Fig. 1). The principle branches are up to 130 microns in diameter. The species is known only from the type collection (M. Doty No. 13,582) made November 13, 1955 by the junior author. The type is deposited in the Bernice P. Bishop Museum with isotypes in the herbaria of the senior author, the New York Botanical Garden, the University of California (UCM-132664), and the University of Adelaide (21,100).

It is to be noted (Figs. 2, 6) that the arching of the branches begins distad of the appearance of branch initials in *C. hawaiiensis* and appears to be correlated with elongations of the first pericentral cell produced. This characteristic of the branches is much like that figured by Silva and Cleary (1954: 252, fig. 15) for *Platysiphonia parva*.

The bases of the apical cells of the young endogenous branches, which are the only branches that become corticated, are about 10 microns in diameter. The bases of the apical cells of the principle axes are often 12 microns in diameter.

The discoidal segments cut off from the apical cells of the main branches elongate until they are at least one-third of their diameter in length (Figs. 2, 5, 6). They then produce abaxially first a dorsal pericentral cell (Figs. 6, 7). At this time there are usually two discoid cells between that which bear this first pericentral cell and the apical cell. It appears that the pericentral cells to the right and left of the dorsal first pericentral cell are the next

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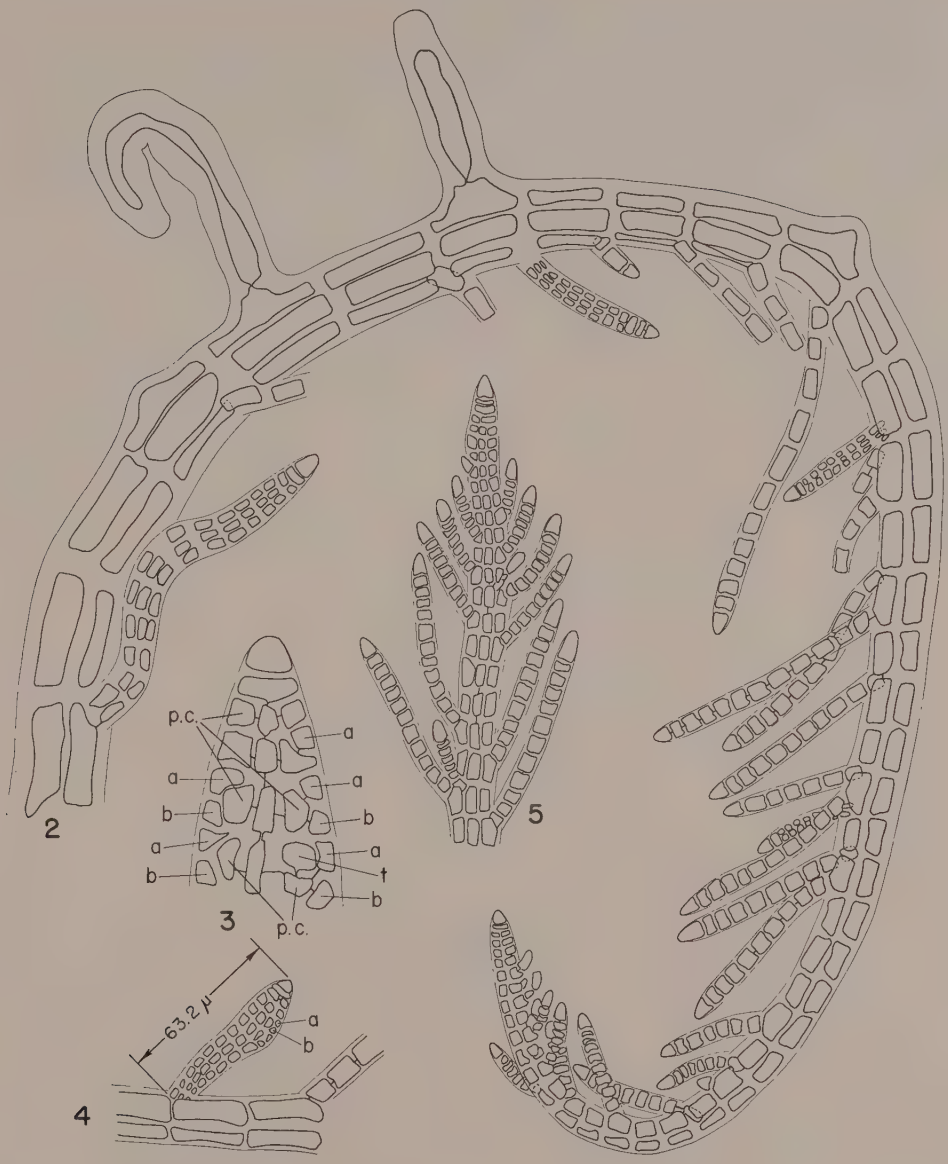
FIG. 1. Sketch of a major branch system showing branch curvature, positions of branches of different kinds, and relative diameters. The largest of the four major branches shown was about 0.5 cm. in length.

to be produced. The fourth pericentral cell is cut off opposite the first and from about the fifth segment removed from the apical cell. Collections of *Cottoniella arcuata* made by A. J. Bernatowicz in Bermuda (53-380 and 53-96) show a similar sequence of pericentral cell development. This is in contrast to what Boergesen (1930: 146) says is true of his *C. fusiformis* from the Canary Islands.

The endogenous branches arise (Figs. 5, 7) from the anterior ventral surfaces of segments in front of or at the corner of the ventral pericentral cells. An endogenous branch on a segment is somewhat parallel to any exogenous branch that may be on the same segment. Endogenous vegetative branches develop entirely like the parent axis and (as in *C. arcuata*) curve toward the parent axis in their development. The lateral pericentral cells on vegetative branches were seen in no case to divide other than in the production of exogenous branches. In more basal parts of the thalli, the longest branches of this sort are up to 675 microns long.



FIGS. 2–5. (2) Cellular detail of one indeterminate branch to show relationships of size and position of ultimate branch kinds and their origins in part. (3) Ventral view of tetrasporangial stichidium apex. Neither dorsal nor ventral pericentral cells shown. (4) Young tetrasporangial stichidium. (5) Cellular detail of indeterminate branch apex from ventral view. Neither dorsal nor ventral pericentral cells shown.

Exogenous monosiphonous branches appear on about the sixth or seventh segment removed from the apical cell (Figs. 2, 5). Boergesen's figure (1919: 336, fig. 335) shows the monosiphonous branches as arising at about the tenth to fourteenth segment in his species, *C. arcuata*. With Bermuda materials of *C. arcuata*, provided by A. J. Bernatowicz, we have been able to confirm this observation. Boergesen also describes and figures the monosiphonous branches as arising on the convex side of the branch endogenously. In the Bernatowicz collections cited, the tips curve and the branches appear on the convex side, the branches then become reflexed or curved in the other direction so that at maturity the branches do indeed protrude from the concave surface.

These uncorticated exogenous branches are about 20 microns in diameter and, while the basal cell hardly becomes more than twice its diameter in length, the other proximal cells of these ultimately 14- to 16-celled branches become up to 5 times as long as broad (Figs. 1, 2). The cells of the distal portion of these more or less determinate branches become progressively shorter distally until they are, just beneath the apical cell, discoid in immature branchlets with hemispherical apical cells, or elongated and tapered toward the slender conical apical cell of a mature branchlet.

The basal cells of the uncorticated exogenous branches are attached by their primary pit to the anterior-ventral corner of a lateral pericentral cell. They occur alternately on the right and left lateral pericentral cells of successive segments (Fig. 5). Secondary pits are formed in abundance between this basal cell and at least the lateral pericentral cell in the next anterior segment (Fig. 8). At least one of these secondary pits may be unusually large (see Fig. 8).

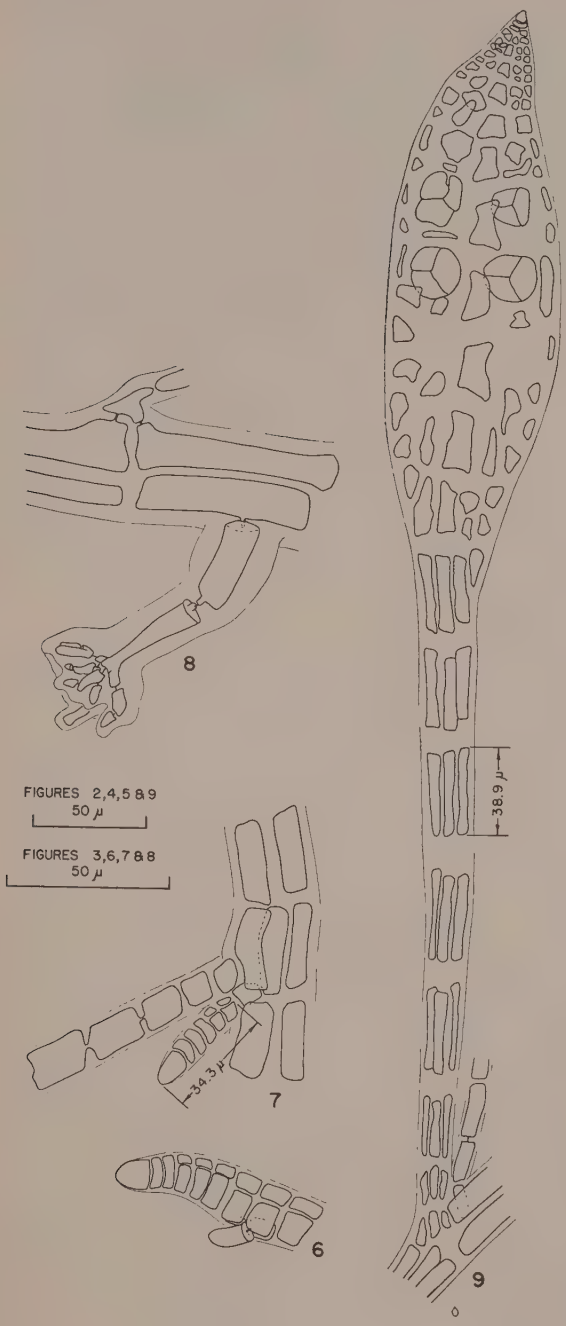
The older exogenous branches apparently drop off as the axes develop. Boergesen (1919: 147, fig. b) notes that exogenous branching was very rare in *C. fusiformis*. He illustrates them, however, as exogenous at least once. According to Papenfuss (1944: 207), of the Delesseriaceae, only *Taenioma* and *Caloglossa* have exogenous branches. On this point our organism seems to be more rhodomelaceous than delesseriaceous.

The rhizoids are exogenous and appear several segments posterior to the level at which endogenous branches appear (Figs. 1, 2, 8). They do not appear until after the other branches have appeared. They appear only from dorsal pericentral cells in our material. Sometimes a segment will bear one of each of the three types of branch. The rhizoids (Figs. 2, 8) become separated from the parent pericentral cell (Fig. 2) at an early age and may become a few cells in length (Fig. 8). There is a tendency for the rhizoids to be curved. Their tips become a disc of rows of small, crowded cells, connected by pits, that in some instances seem to have arisen by sympodial development (Fig. 8). In at least one case this pad was stuck to the *Polysiphonia* with which it was found entangled.

The tetrasporangial stichidia arise endogenously in the same positions as do endogenous vegetative branches and appear to take their place. The discoid segments cut off by the apical cell of the stichidium usually elongate to about one third of their diameter before cutting off a pericentral cell (Figs. 3, 4, 9). This usually occurs at the level of the first or second discoid cell. The first pericentral cell appears to be either dorsal or ventral; each situation was found with about equal frequency. Only a few rather disarranged branches were available for study of this point, however. The other three pericentral cells appear very rapidly and are usually present on the second or third discoid central cell segment.

The younger pairs of tetrasporangia arise towards the apex of the stichidium (Figs. 3, 9). The lumen of the tetrasporangium is radially elongated at least to a length of 60 microns at maturity, while the diameter parallel to the stichidial axis is about 47 microns. The tetraspores (Fig. 9) are tetrahedrally arranged.

The lateral pericentral cells each serve as



FIGS. 6–9. (6) Indeterminate branch apex from lateral view. Central cells not shown beyond third cell below apex, ventral pericentral cell not shown in lowest segment. (7) Cellular details of young indeterminate branch. (8) Cellular details of mature rhizoid and of an exogenous determinate branch basal cell. (9) Outline of mature tetrasporangial stichidium which had shed the contents of two sporangia.

tetrasporangial mother cells (Fig. 3*pc*). They produce two "cover" cells (*a* and *b* in Figs. 3, 4) (apical and basal), the tetrasporangium (Fig. 3*t*), and finally a third cover cell somewhat basally. The first two laterally produced cover cells curve around over the tetrasporangium. The third is produced ventrally and, likewise, grows over the surface of the sporangium. Thus the two pericentral cells (dorsal and ventral) and the three cover cells cut off by each of the two lateral pericentral cells at each side (i.e., 8 cells in all) make up the outer layers of each fertile mature stichidial segment.

DISCUSSION

Since "cortication" in our material consists, in sterile regions, only of pericentral cells, the opinion becomes critical as to whether or not there are "flanking cells" or "cover cells" present, cut off by the pericentral cell before the tetrasporangium. If there are flanking cells, one is inclined to consider *Cottoniella hawaiiensis* a member of the Delesseriaceae, accepting Papenfuss's (1944: 202) statement that, in that family, cover cells are cut off only after the tetrasporangium.

Certainly one "weakly-covering" cell is cut off *after* the tetrasporangium, and the lateral pericentral cells produce two cells lateral to themselves *before* the tetrasporangium is produced in the same way the flanking cells are produced in *Platysiphonia*.

The tetrasporangia are somewhat more covered over in *C. hawaiiensis*, and by a more complicated cover cell system, than in the case of *Taenioma* (Papenfuss, 1944: 195, figs. 4, 14, 199) or *Platysiphonia* (Silva and Cleary, 1954: e.g., 256, fig. 27), and other similarly simple members of the Ceramiales. However, the stichidial features are shown here for *Cottoniella* and for tetrasporangial areas of other genera, by various authors elsewhere, to be quite homologous in structure. Schotter has emphasized already (1951: 287) the similarity between the vegetative structure of *Cottoniella* and these two other genera. Especially are *Cottoniella* and *Platysiphonia* similar in basic vegetative structure.

As regards the sequence of pericentral cell formation, it is interpreted as rhodomelaceous in all the *Cottoniella* material we have seen, following Naegeli's (1847, fide Schotter) early distinction of this family from the Delesseriaceae on this point. Boergesen's figures (1919: 147, fig. 59b, c) lead us to expect this in *C. fusiformis*, despite his statements to the contrary.

All in all it appears that *Cottoniella* is one of those simple algae that would some time ago have been placed in the Sarcomenioideae (Sarcomenieae of authors). The flattened four pericentral-celled nature of the stichidia of *C. hawaiiensis* bearing two tetrasporangia per segment bespeak of a delesseriaceous affinity for *Cottoniella*, and we are inclined to relegate it at present to that family but recognize its possible connecting-link nature between that family and the Rhodomelaceae.

SUMMARY

Study of tetrasporic *Cottoniella hawaiiensis*, a previously undescribed species from Hawaii, indicates placement of this genus among the Delesseriaceae on the basis of the mode of tetraspore production, or intermediately between that family and the Rhodomelaceae on the basis of weighting and interpretation of other features such as flanking cells, cover cells, apical cell development, and exogenous branches. The appearance of exogenous branches as a regular feature and the degree of development of the pericentral cell systems set the Hawaiian species apart from all others in this genus.

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Hawaiian Helminths 1. *Trigonocryptus conus* n. gen., n. sp. (Trematoda: Fellodistomidae)[1]

W. E. MARTIN[2]

THIS WORK was done at the Hawaii Marine Laboratory, University of Hawaii. Twenty-nine balloon fish, *Tetraodon hispidus* L., collected by trap, were examined for helminths. Four harbored in their intestine a total of eight trematodes which do not fit into any genus or species thus far described.

The worms were killed in cold Heidenhain fixative under light cover glass pressure, stained in celestine blue B, and mounted in H.S.R. Microscopic Mounting Medium.

All measurements are expressed in millimeters.

TRIGONOCRYPTUS n. gen.

GENERIC DIAGNOSIS: Fellodistomidae: Small conical trematodes with ventrolateral body folds separated posteriorly and converging anteriorly. Posterior end of body bears a short velum laterally and dorsally. Inverted tail cone present. Cuticula relatively thick, spined. Oral sucker subterminal with ventrolateral extensions. Ventral sucker large, transversely elongate and ridged, indented anteriorly and posteriorly. Prepharynx and esophagus very short. Pharynx oval to spherical. Ceca short, saccular, not extending posterior to mid-body. Testes paired, opposed, oval, near posterior end of body. Cirrus sac oval to nearly spherical, immediately anterior to or slightly overlapped by acetabulum, encloses a weakly bipartite seminal vesicle, a well-developed prostate and a short cirrus. Ovary oval, median, partly between to a short distance anterior to testes. Seminal receptacle near, usually smaller than, ovary. Mehlis gland beside ovary, inconspicuous. Vitelline follicles in two major groups on each side of body extending from anterior acetabular to posterior oral sucker levels. Uterus mainly confined to posterior half of body, between and anterior to testes. Uterus and cirrus use common genital atrium which opens to outside medially and ventral to gut bifurcation. Eggs oval, numerous, operculated, with tiny knob at anopercular end. Excretory bladder U-shaped. Parasitic in the intestines of marine fish.

GENOTYPE: *Trigonocryptus conus*.

Trigonocryptus conus n. sp.

Figs. 1–5

SPECIFIC DIAGNOSIS: With characters of genus. Body conical and pink in life, triangular when fixed under pressure. Cuticle 0.012–0.016 thick, armed with spines. Spines approximately 0.017 long with shouldered peripheral terminations (Fig. 4). Body 1.37–1.82, average 1.58 long and 0.98–1.4, average 1.24 wide. Oral sucker subterminal, muscular, with folds meeting those of the body wall, 0.39–0.55,

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Southern California, NR 165–252. The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

I wish to express my appreciation to Drs. A. H. Banner, R. W. Hiatt, and L. D. Tuthill of the Hawaii Marine Laboratory and the Department of Zoology and Entomology, University of Hawaii, for the use of space and equipment during my sabbatical leave.

Contribution No. 107 Hawaii Marine Laboratory, University of Hawaii, Honolulu. Manuscript received March 4, 1957.

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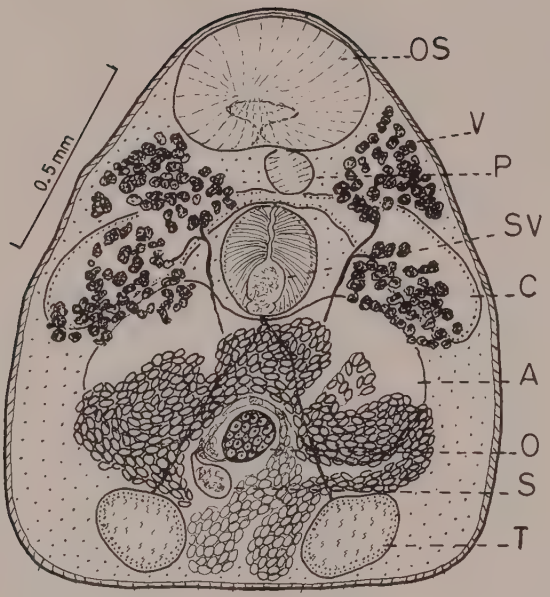

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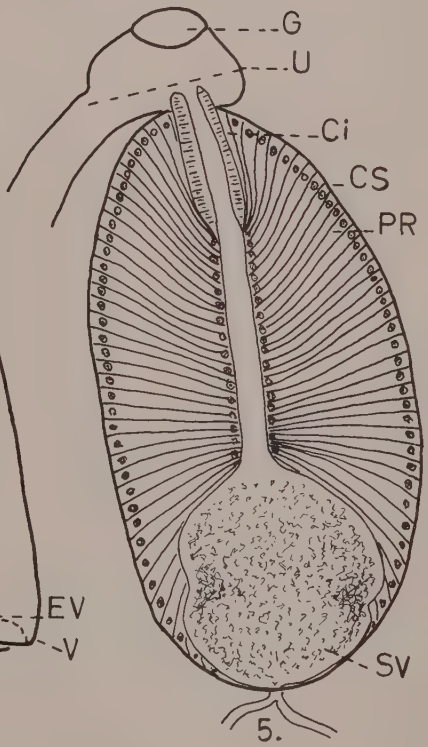

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average 0.44 anteroposteriorly and 0.41–0.55, average 0.48 transversely. Ventral sucker large, muscular, indented anteriorly and posteriorly, with two or three transverse ridges, 0.45–0.59, average 0.52 anteroposteriorly and 0.64–1.02, average 0.86 transversely. Prepharynx short, pharynx 0.093–0.112, average 0.10 anteroposteriorly and 0.081–0.121, average 0.11 transversely. Esophagus short, ceca thin walled, short, saccular, not extending beyond body equator. Testes near posterior end of body, opposed, oval, 0.17–0.28, average 0.22 long and 0.14–0.21, average 0.17 wide. Vasa efferentia join just before reaching cirrus sac. Cirrus sac median, immediately anterior to or partially overlapped by acetabulum, containing weakly bipartite seminal vesicle filled with sperm, a well-developed prostate, and a short cirrus which may project into the genital atrium. Genital pore median and ventral at level of gut bifurcation. Ovary median, usually a short distance anterior to testes, 0.096–0.17, average 0.15 long and 0.084–0.15, average 0.12 wide. Seminal receptacle pyriform, usually a short distance posterior and to the right of the ovary. Mehlis gland close to ovary, inconspicuous. Vitelline follicles in two major clusters on each side of body between anterior acetabular and oral sucker levels. Common vitelline duct not expanded to form reservoir. Uterus after leaving ovary passes posteriad to loop between the testes then extends anteriad to loop transversely and fill much of the acetabular region of the body. It then passes anteriorly to the genital atrium. The uterus of mature worms is filled with eggs. Newly formed eggs colorless, older eggs brown. Eggs 0.037–0.046, average 0.041 long and 0.022–0.025, average 0.023 wide, operculated, with a small knob at anopercular end. Excretory vesicle U-shaped, thin walled, with pore opening medially and subterminally.

TYPE SPECIMEN: *Trigonocryptus conus*, deposited as number 55285 in the U. S. National Museum, Helminth Collection.

HOST: *Tetraodon hispidus* L., balloon fish, in intestine.

LOCALITY: Kaneohe Bay, Oahu, Hawaii.

DISCUSSION

The genus *Trigonocryptus* most closely resembles *Paradiscogaster* Yamaguti, 1934. The major difference is in the vitellarian distribution, in one cluster on each side of the body confined to the cecal region in *Paradiscogaster*, and in two clusters on each side of the body extending from the acetabulum to the oral sucker in *Trigonocryptus*. Other differences are the ventrolateral extensions of the oral sucker in *Trigonocryptus* while this organ in *Paradiscogaster* has the usual circular outline; the unusual body shape in *Trigonocryptus* with a ventral, triangular depression, a velum on the lateral and dorsal margins of the posterior part of the body, and a truncated posterior end while the body shape is pyriform in *Paradiscogaster*. Yamaguti (1938) described a small tail cone in *Paradiscogaster chaetodontis*. *Trigonocryptus conus* has an inverted tail cone, dorsal to the excretory pore, which was never seen everted even in active worms. *Paradiscogaster piriformis* Yamaguti, 1934, was found in *Pleuronichthys cornatus* in Japan (Yamaguti, 1951); *P. chaetodontis* Yamaguti, 1938, in *Chaetodon collaris* in Japan and *C. strigangutus* and *C. trifasciatus* in Okinawa (Yamaguti, 1953); and *P. caranxi* (Srivastava, 1939) Yamaguti, 1953, in *Caranx kalla* in India. Although a goodly number of Hawaiian chaetodons and a few carangids have been examined, no species of *Paradiscogaster* or *Trigonocryptus* have been found in them. Hanson (1955) has reported a related form, *Discogasteroides ha-*

FIGS. 1–5. *Trigonocryptus conus:* 1, Dorsal view; 2, ventral view; 3, egg; 4, body spine; 5, terminal genitalia. A, Acetabulum; C, cecum; Ci, cirrus; CS, cirrus sac; EV, excretory vesicle; G, genital pore; O, ovary; OS, oral sucker; P, pharynx; PR, prostate; S, seminal receptacle; SV, seminal vesicle; T, testis; U, uterus; V, vitellaria; V, velum. All drawings made with the aid of a camera lucida.

waiensis, from a Hawaiian trunk fish, *Ostracion sebae* Bleeker.

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THE ROLE OF THE BLOOD IN THE TRANSPORTATION OF STRONTIUM^{90}–YTTRIUM^{90} IN TELEOST FISH

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THE ROLE OF THE BLOOD IN THE TRANSPORTATION OF STRONTIUM^{90}–YTTRIUM^{90} IN TELEOST FISH [1, 2]

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As the result of global fallout and the introduction of radioactive wastes from nuclear reactor plants into the oceans, marine organisms are being subjected to an environment which is potentially hazardous to themselves and to other members of the ecosystems involved. During the last few years, a study has been made in this laboratory of various aspects of the metabolism of radiostrontium by marine fish. These fish may pick up strontium directly from sea water, by way of the skin, gills, or by swallowing the water (Boroughs, Townsley and Hiatt, 1956). They may also take up this element from their food. In any event, the transportation of strontium within the fish, including its excretion, depends upon its transportation by the blood, except for the strontium which is unabsorbed from the digestive tract.

It is the purpose of this paper to report on certain aspects of the transportation of strontium^{90}-yttrium^{90} in teleost blood.

Materials and Methods

The species used in this experiment was *Tilapia mossambica,* a teleost fish. Individuals weighed between 50 and 110 grams each. They were kept in tanks supplied with running sea water.

Two concentrations of Oak Ridge Sr^{90}-Y^{90} were prepared by dilution with saline solution approximately isotonic with *Tilapia* blood. Those fish which were to be bled a day or more after injection were given 100μc of Sr^{90}, while the fish killed at shorter time intervals were given only 10 μc. In both instances the dose injected was 0.2 ml.

The injections were made, and blood was withdrawn with the fishes' opercula in water. Separate fish were used for each time interval studied instead of using a single fish for repetitive bleedings. All the fish were handled as gently and uniformly as possible, and their eyes were covered with the hand. We believe this procedure results in a minimum of trauma.

The Sr^{90}-Y^{90} dose was injected directly into the ventricle of the heart. At predetermined time intervals of 5, 15, 30, and 45 minutes and 1, 4, and 8 days, as much as possible of each fish's blood was withdrawn through the kidney sinus. A red blood cell count was made each time a fish was injected and again when blood was removed.

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Immediately after removing the blood from the fish, triplicate 0.1-ml. samples were pipetted onto circles of one thickness of absorbent tissue on aluminum planchettes. Three-tenths-ml. aliquots of the remaining blood were centrifuged for 10 minutes at 2100 rpm in calibrated small bore hematocrit tubes in an International clinical centrifuge. The separated blood in one tube was used for measuring the radioactivity in the plasma and also that associated with the cells. From a second tube the plasma was removed without disturbing the packed cells. Five-hundredths ml. of these cells were washed by re-suspending them twice in fresh saline solutions. All the saline washings were pooled. In a third tube, the same volume of saline-washed cells was lysed with distilled water. The ghosts were washed with distilled water until no further radioactivity could be removed from them. The lysing solution containing the cell contents was added to the distilled water wash for measurement of the radioactivity of the cells exclusive of that bound to the stroma.

Separated organs and tissues were ashed and prepared for counting as previously described (Boroughs, Townsley and Hiatt, 1956). Radioactivity was measured with a thin window G-M tube using a commercial scaler. Counts were corrected for coincidence whenever necessary.

In order to get an approximation of mixing time, Sr^{85} was injected in the heart. Ten, 20, and 30 minutes later, blood was removed from the ventral aorta and from the kidney sinus, and 0.1-ml. samples were counted in a well scintillation counter with the aid of a single channel pulse height analyzer.

Results and Discussion

Preliminary experiments

Since very little is known about fish blood, we were at the outset faced with problems which were not pertinent to the main idea of this research. The first problem to be overcome was the bleeding, because apparently very few biologists have successfully removed blood directly from teleost fish (Prosser, personal communication). In general, fish have been bled by cutting the tail and allowing the blood to drip. Even more refined methods have involved the use of heparin, citrate, or other anticoagulants. We have found it difficult to withdraw unclotted blood from *Tilapia* if the fish had been kept out of water for even a short time. There is probably a dehydration of the blood in some species of fish as a result of asphyxiation (Hall, Gray and Lepkovsky, 1926). If *Tilapia* were stressed by prolonged chasing with a net, by rough handling or by repeated bleeding, removal of blood was difficult even though they were not taken from the water. The cell/plasma ratio increased as it did with asphyxiation.

We had previously observed red blood cell counts which varied between 1 and $4 \times 10^6/mm^3$ in this species of fish, and other workers (Young, 1949) have observed similar large variations with other teleost fishes. Table I is a summary of the rbc counts of the fish used in this experiment and shows that these variations are not intrinsic and that it is possible to remove fish blood that has a reasonably small fluctuation in the rbc count. This blood does not clot even on prolonged standing at room temperature.

The tremendous shift in the number of red blood cells observed in fish blood

TABLE I

Red blood cell count in Tilapia mossambica

Time interval between injection and killing	RBC/mm.³ of blood	
	Counted before dose injected	Counted before blood withdrawn
5 min.	1.444×10^6	1.627×10^6
5 min.	1.150×10^6	1.423×10^6
5 min.	1.375×10^6	1.400×10^6
15 min.	1.350×10^6	2.050×10^6
15 min.	1.209×10^6	1.374×10^6
30 min.	1.548×10^6	1.525×10^6
30 min.	1.175×10^6	1.400×10^6
45 min.	1.125×10^6	1.460×10^6
1 hr.	1.200×10^6	1.600×10^6
1 hr.	1.162×10^6	1.384×10^6
1 hr.	1.050×10^6	1.025×10^6
2 hr.	1.223×10^6	1.347×10^6
2 hr.	1.148×10^6	1.326×10^6
2 hr.	1.150×10^6	1.220×10^6
4 hr.	1.151×10^6	1.169×10^6
4 hr.	1.209×10^6	1.137×10^6
8 hr.	1.199×10^6	—
8 hr.	1.011×10^6	1.102×10^6
1 day	1.312×10^6	1.396×10^6
1 day	1.649×10^6	1.598×10^6
2 days	1.100×10^6	1.199×10^6
2 days	1.298×10^6	1.298×10^6
4 days	1.103×10^6	—
4 days	1.271×10^6	1.362×10^6
8 days	1.150×10^6	1.273×10^6
8 days	1.018×10^6	1.175×10^6

could mean that the plasma, or some portion of it, either leaves the circulatory system or is in effect removed by some pocketing device. The increase in red blood cells may also result from the introduction into the blood stream of cells previously sequestered in an organ or tissue. Studies on fish blood volume and mixing time using either classical techniques or radioisotopes would be of little value if the fish were stressed.

The circulation of fish blood is distinguished from that of higher animals in that oxygenated blood does not necessarily return to the heart. All the blood from the heart goes to the gills, but from the gills the blood may go to the head,

TABLE II

The mixing time of Tilapia blood

Blood source	Minutes elapsed	Counts/min.
Ventral aorta	10	249
Ventral aorta	20	79
Ventral aorta	30	51
Kidney	10	40
Kidney	20	45
Kidney	30	50

Dose: 8477 cpm in 0.2 ml. injected into ventricle of heart.

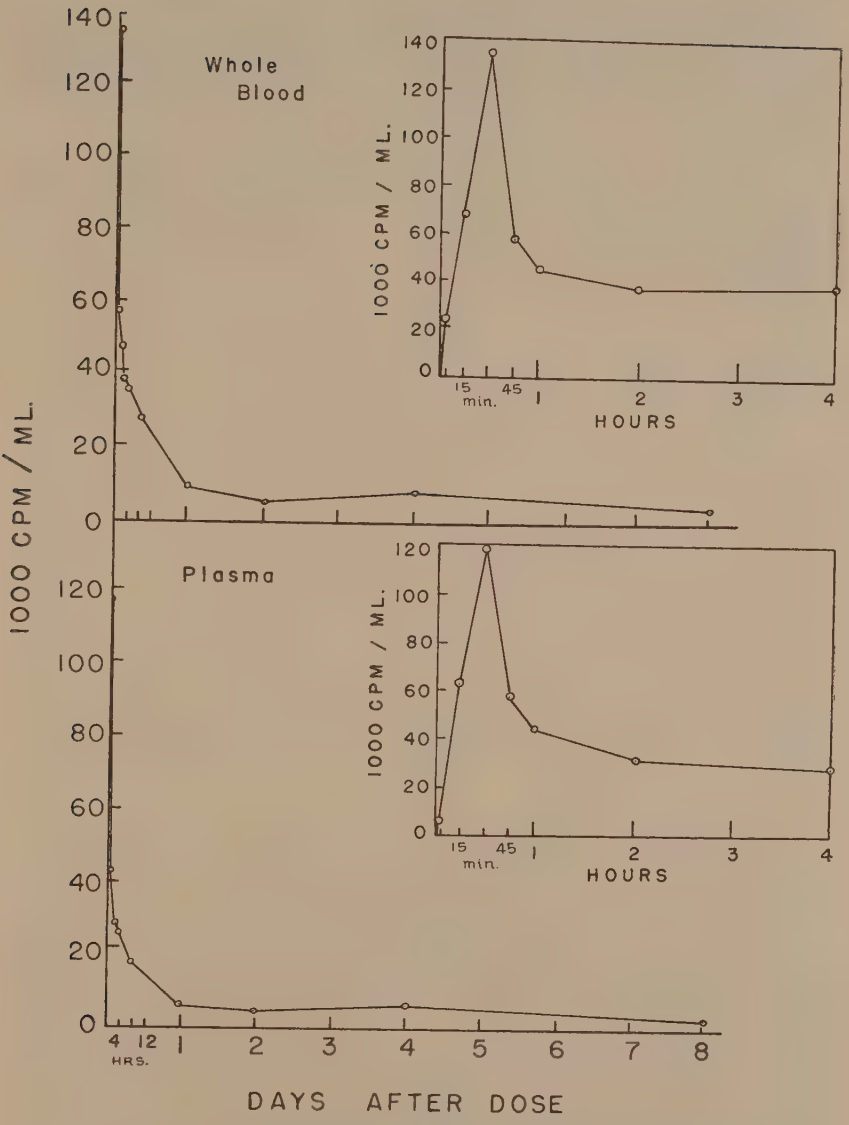

FIGURE 1. The disappearance of Sr^{90}–Y^{90} from the whole blood and plasma of *Tilapia mossambica*.

back to the heart, or to the remainder of the body. This means that mixing is a more complicated process in fish than it is in the higher animals.

The results of studying mixing time in a single fish are shown in Table II. It can be seen that the bulk of the Sr^{85} injected into the heart remained in the anterior portion of the fish, and that it required about 30 minutes for the blood from the ventral aorta and that from the kidney to reach the same level.

Since we lack precise information about blood volume, we have assumed that it is roughly proportional to body weight. We have done this not only on the basis of our own work, but because Martin (1950) has suggested a similar relationship for other teleost fishes.

Rate of disappearance of Sr^{90}-Y^{90} from the blood

Figure 1 shows the rate of disappearance of Sr^{90}-Y^{90} from whole blood and plasma. The numbers have been corrected for body weight. The activity is given in counts/min./ml. whole blood and cpm in the plasma present in 1 ml. of whole blood. Each point on the curve represents the average activity from at least two fish. It can be seen that practically all the radioactivity in the whole blood is carried in the plasma, and that the formed elements can be responsible for only a very small amount. The two curves are practically superimposable. The small inserts on this graph show the appearance of radioactivity during the first few hours, and the larger graph extends the curves to 8 days. Since all the radioactivity was injected into the heart at zero time, at first glance it may seem odd that the amount of radioactivity recoverable from the blood *increases* up to 30 minutes. However, Table II indicates that this apparent increase is a reflection of the mixing time. At least two processes are occurring during this time which make it extremely difficult to find out exactly how much radioactivity is in the blood system. First, the isotopes are being excreted as soon as they appear in the blood, at first principally by way of the gills. Second, radioactivity is rapidly accreted by the various organs and tissues, and thus the concentration is decreasing continuously. We would like to emphasize that it is the resultant of these processes that is being measured.

The radioactivity was very rapidly lost from the blood during the next 30 minutes, and after 24 hours, only between 0.8 and 1.6 per cent of the injected dose remained in the blood, assuming a blood volume of 2–4 per cent of the body weight. The shape of the curves shows that more than one rate process is involved in the disappearance of the radioactivity from the blood. It must be emphasized at this point that the above samples were counted at least three weeks after the fish was killed, so that we were observing the radioactivity in an equilibrium mixture of Sr^{90}-Y^{90}. Strontium^{90} has a half-life of about 28 years and a maximum beta energy of 0.61 Mev. It decays to form radioactive Y^{90} which has a half-life of 2.54 days and a maximum beta energy of 2.18 Mev. Secular equilibrium exists when the Y^{90} decays as fast as it is formed, and the radioactivity of such a mixture is the sum of the radioactivity of the separate isotopes.

In an equilibrium mixture, therefore, no decay of radioactivity would be observable during this experiment unless the two isotopes were separated by either biological or physico-chemical processes. Such a fractionation can be detected by following the counting rate of a sample daily. No changes in this rate will be

observed if no fractionation has occurred. If the rate increases, Y^{90} has been removed and is building up to its equilibrium value at which point it will level off. If the rate decreases, the bulk of the radioactivity must be due to the Y^{90} which is decaying, and the counts will decrease until a level is reached which is a function of the amount of Sr^{90} present.

The role of the blood fractions in the transport of Sr^{90}-Y^{90}

The increase in the counts/minute of the whole blood and plasma in Figure 2 is due to the build up of Y^{90}. There are two simple explanations for the loss of yttrium from the blood. One is that the yttrium was lost prior to its appearance in the blood initially, that is, adsorbed to the glassware used in making the dilutions

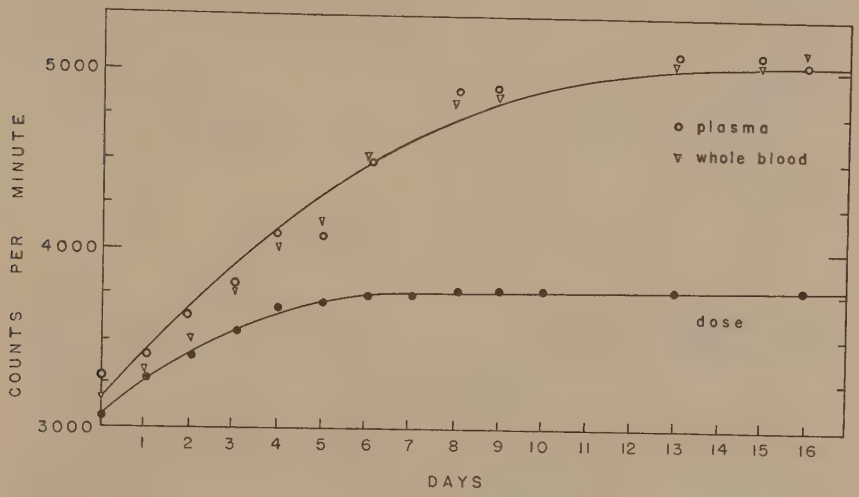

FIGURE 2. The increase with time of radioactivity in samples of whole blood, plasma, and the dose, indicating the build-up of Y^{90}.

and injections. The second explanation is that the yttrium was lost to various organs and tissues through which the blood passed. These explanations are not mutually exclusive and we believe that both processes occur.

In Figure 3, the curve labelled "dose" was obtained by counting planchettes prepared from the Sr^{90}-Y^{90} present in the syringe used for injections. It can be seen that over a period of time, the cpm increased, indicating that some Y^{90} was lost from the equilibrium mixture. This Y^{90} was lost to the glassware. The curve for whole blood and plasma, however, increased to a much higher value, indicating that additional Y^{90} had been removed after the dose was injected.

Figure 3 shows the rate of radioactive decay of the washed and unwashed cells, the saline washings, the washed ghosts, and the distilled water washings which include the cell contents. The decay of the unwashed cells suggests that both Sr^{90} and Y^{90} were associated with the cells. The decay of the washed cells, saline

wash, and ghosts, however, suggests that the Sr^{90} is readily removable either from or through the cell wall. The activity remaining in the washed cells and ghosts indicates it to be Y^{90}, because the decay rates are very similar to the rate for pure Y^{90}. All these conclusions are in harmony with the findings of Thomas *et al.* (Thomas, Litovitz, Rubin and Geschickter, 1950), who showed that radiocalcium, metabolically similar to strontium, was carried in the plasma of rabbit blood.

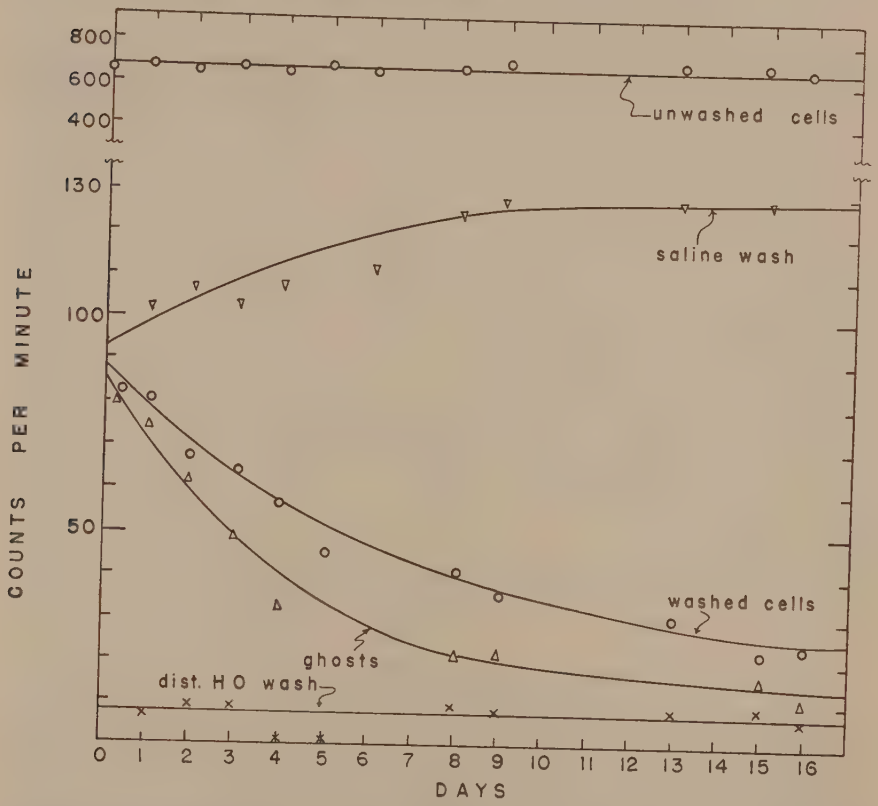

FIGURE 3. The radioactive decay of washed and unwashed cells, the saline and distilled water wash, and the cell ghosts. The decay of washed cells and ghosts indicates that they pick up Y^{90} rather than Sr^{90}.

Retention and distribution of Sr^{90}-Y^{90}

Figure 4 shows the retention by the fish of the injected Sr^{90}-Y^{90} as a function of time. The upper curve represents the entire fish, and the other curves represent, respectively, the bone, integument, gills, muscle, and visceral organs. Each point is the average of at least two fish, and the samples were counted at secular equilibrium. These results may be compared with those obtained previously by

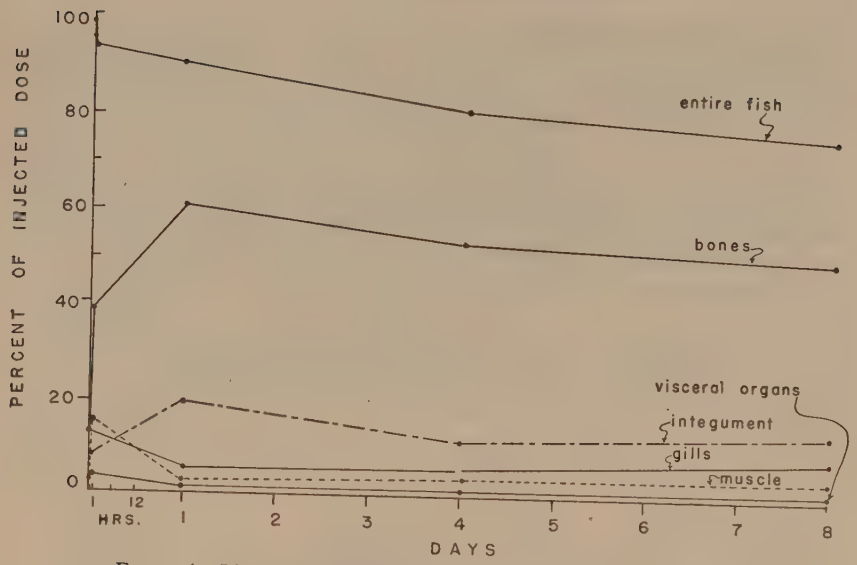

FIGURE 4. The internal distribution of Sr^{90}–Y^{90} injected in the heart of *Tilapia mossambica*.

Boroughs *et al.* on the retention of Sr^{89} by *Tilapia* following ingestion or intramuscular injection. In all instances, the rank order of the percentage of dose retained is the same as that in Figure 4, indicating that once the strontium is absorbed by the blood system, its internal distribution is the same.

Table III emphasizes the rapidity with which the radioactivity appears in the various tissues, including the bones. A small amount of vascular tissue and blood is dissected with the bones, but this additional radioactivity is obviously a very small percentage of the total in the bones. This appearance and retention in the bones cannot be due to accretion by growth, but must represent simply an exchange reaction.

TABLE III

Retention and internal distribution of Sr^{90}–Y^{90} after intracardial injection

Time interval	% injected dose remaining (samples at secular equilibrium)					
	Total of fish*	Bones	Integument	Gills	Muscle	Visceral organs
5 min.	98.2	13.2	13.4	13.4	3.1	3.4
15 min.	95.9					
30 min.	93.2	39.3	8.2	17.3	11.9	4.0
1 day	92.1	61.7	19.2	4.9	4.5	1.4
4 days	81.5	53.7	17.1	4.7	4.2	1.4
8 days	76.6	50.2	16.2	7.4	3.3	1.4

* Including blood.

Biological fractionation of Sr^{90}-Y^{90}

Three fish were injected with Sr^{90}-Y^{90} and killed five minutes, 30 minutes, and one day later. Since the amount of separation of the two isotopes by the glassware was unknown, it is not possible to draw a curve showing the rate of decay of the radioactivity in the various organs that would be a true measure of the decay due to the fractionation by the organs themselves. The planchettes were counted one day after the fish were killed, and this value was taken as a base line. They were then counted until secular equilibrium had been re-established. Table IV shows the percentage increase or decrease in radioactivity in the various organs with respect to the radioactivity present at one day.

TABLE IV

Fractionation of intracardially injected Sr^{90}–Y^{90} by organs and tissues of Tilapia mossambica

Organ or tissue sample	% Decrease of activity 1 day to secular equilibrium	Organ or tissue sample	% Increase of activity 1 day to secular equilibrium
Liver	53.1	Gills	73.8
Gall bladder	42.5	Stomach	22.3
Heart	38.6	Brain	18.3
Kidney	18.8	Muscle	16.3
Spleen	10.6	Intestine	10.4
Gonads	8.5	Eyes	6.9
		Urinary Bladder	5.6
Urine	28.5	Skin	5.0
Blood clots	26.8		
Scales	14.1		
Fat	11.4	Feces	2.1

It can be seen that the first two columns represent the organs which concentrated Y^{90} more than they did Sr^{90}, while the last two columns represent organs that favored the Sr. In general, the more vascular organs and tissues preferred yttrium.

SUMMARY

1. Blood can be easily removed without clotting from the heart or kidney sinus of fishes if the fish are handled gently and their opercula are kept immersed.

2. Blood so removed has a uniform number of red blood cells/mm³.

3. The mixing time of Sr^{90}-Y^{90} injected in the ventricle of *Tilapia mossambica*, a teleost fish, is approximately 30 minutes.

4. Sr^{90}-Y^{90} rapidly disappears from the blood. At 24 hours, only between 0.8 and 1.6 per cent of the injected dose remains in the blood.

5. The disappearance of radioactivity from the blood depends on more than a single process.

6. Almost all of the Sr^{90} in whole blood is carried by the plasma.

7. Very little Sr^{90} is found either in the cells or on the cell walls.

8. $Yttrium^{90}$, on the other hand, is present in the stroma.

9. The pattern of internal distribution of intravascularly injected Sr^{90}-Y^{90} is the same as that which was found for either intramuscular or oral administration in the same species.

10. Vascularized tissues have a greater avidity for Y^{90} than they have for Sr^{90}.

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1958, v98, 776-778

Observations on Toxins of Poisonous Fishes.* (24182)

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Certain fishes of the tropical Pacific, including fish as diverse as puffers (*Tetraodon*) and snappers (*Lutjanus*), have long been reported to be highly toxic when eaten. Hiyama(1), by feeding tests, and Halstead and Bunker(2), by intraperitoneal injections into mice of aqueous extracts of fish viscera and flesh, have confirmed these reports. This toxin is not the result of bacterial decomposition, but occurs in the flesh of fresh fish. These toxins may vary among the different species(3). The object of these tests was to explore the nature of the toxin sufficiently to permit planning of future studies on poisoning characterized by Halstead(3) as *Ciguatera*. Fish producing this syndrome are reported by islanders to be highly toxic in certain restricted areas, but elsewhere are excellent as food. To avoid any confusion of toxins that could be different, only body muscles of *Lutjanus bohar* Forskal from Palmyra were used.‡ About 20 fish were tested ranging 14 to over 24 inches long. Only fish longer than 16 inches produced the syndrome characteristic of *Ciguatera* poisoning.

Results. The first phase was to find an animal that would respond consistently with symptoms paralleling those found in man. Cats so responded, as reported by others, but were not readily available. A variety of other vertebrates was then tested, but none except the imported mongoose (*Herpestes javanicus auropunctatus*) responded to dosages, in terms of body weight, that caused paralysis and death in cats. The symptoms of the mongoose, like those of the cat, included loss of certain reflexes, for example, failure to withdraw the foot when touched lightly. A progressively severe loss of coordination followed in first the back and then the forelegs which, in acute cases, would prevent the mongoose from standing or righting itself after falling. Death, preceded by coma and

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‡ We wish to thank the U. S. Fish and Wildlife Service for catching the fish which were frozen immediately after capture.

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Cheyne-Stokes breathing, would occur within 36 to 48 hours, if fish of sufficient toxicity was fed. Unlike cats and man, the mongoose never vomited, thereby permitting a more accurate assessment of the amount of fish eaten.

In tests reported below the mongoose was used exclusively; no mongoose, after once showing symptoms, was used again. Mongooses were fed raw fish to determine approximate lethal dose. No fish was found to be lethal under 5% body weight, and no fish was used that did not cause death at 15% body weight. When fluid extracts of the fish were used, they were concentrated and evaporated on Gaines dog food for feeding.

Feeding tests confirmed that toxin in fish is not noticeably reduced by drying the minced muscle at 102°-108°C for 24 hours, or by freezing the whole fish for periods up to 6 months.

Solubilities of the toxin were explored. The implication of test reported by Halstead(2,4) is that the toxin is soluble in distilled water. Ground muscle, thrice extracted with its own weight of distilled water for 24 hours, showed no loss in toxicity, but the extract, concentrated on dog food, caused no reaction when fed.

When dehydrated ground flesh was extracted with chloroform, diethyl ether, or hot absolute alcohol, neither the extract nor residue fed at twice the lethal dosage produced obvious reactions. When dried meat was extracted with 90% ethanol, all of the toxin appeared in the extract; 70% ethanol appeared to be less efficient, and absolute alcohol extracted almost no toxin. The 90% ethanol extract decreased in toxicity when it was evaporated to dryness without dog food.

When secondary extraction of the 90% ethanol solution was made with repurified ether, dioxane, chloroform or petrol ether, no observable toxicity was left in either fraction. However, under an atmosphere of commercial nitrogen (about 3% oxygen) toxicity of extracts was diminished but not lost. When the alcoholic solution was evaporated to dryness under nitrogen, then extracted with petrol ether and the undissolved residue redissolved in 90% ethanol, both fractions gave toxic symptoms. When the experiment was repeated with chloroform or diethyl ether, toxicity was observed only in the chloroform and diethyl ether solutions.

The reliability of Halstead's technics(2,4) was also investigated. Halstead homogenized portions of fish, *i.e.* muscles, viscera, etc., in water, centrifuged the slurry for 25 minutes at 2,000 rpm, and injected 1 ml of supernatant fluid intraperitoneally in 15 to 25 g mice.

Halstead recognized 3 types of mouse reactions to poisonous fish: *weakly positive,* including lacrimation, diarrhea, and ruffling of hair, but the animal recovered; *moderately positive,* including same symptoms, but the animal died within 36 hours; and *strongly positive,* where test animal died within one hour.

Mice showed no symptoms when fed toxic fish even at twice the dose in percentage of body weight that was lethal to a mongoose. An extract was prepared according to the method of Halstead and Bunker(2,4) of a lethally toxic fish and of a non-toxic tuna; these extracts were injected into mice weighing from 20 to 25 g. The 2 mice receiving the tuna injection showed no reaction, but the 2 receiving the lethal fish displayed what Halstead called a "weakly positive" reaction, with slight lacrimation, diarrhea, and ruffled fur.

The extract prepared by this technic was not a solution but a milky suspension. Since mice injected with the most toxic *Lutjanus* available did not die, a fish classified by Halstead as having a different type of toxin was used to determine whether the toxin was in solution or suspension. Liver and gonads of the puffer [*Arothron hispidus* (L.)] were prepared by Halstead's technic; after centrifugation the extract was divided into 2 portions. One portion was filtered twice through filtered paper and finally through a Millipore glass filter. Two mice, weighing between 20 and 25 g, were each injected intraperitoneally with one ml of this solution, while the unfiltered extract was injected into 2 similar mice. The 2 mice receiving the *unfiltered* extract

died but the ones injected with the filtered extract showed no symptoms.

Summary. 1. Not all animals are sensitive to the *Ciguatera*-type toxin. 2. This toxin is not water soluble, but is soluble in 90% ethanol and certain other solvents. 3. The toxin may be detoxified when concentrated in normal atmospheric oxygen, but remains toxic under nitrogen. 4. The test previously used to assess this toxicity is not sufficiently sensitive and depends upon the suspended matter in the extract.

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Pacific Area Distribution of Fresh-Water and Marine Cercarial Dermatitis[1]

GEORGE W. T. C. CHU[2]

CERCARIAL DERMATITIS, a form of "swimmer's itch," is a skin infection caused by the penetration of schistosome cercariae. The disease is also known as schistosome dermatitis. The clinical symptoms start with an itching sensation, which is followed by the development of a rash or petechiae, and in many hypersensitive persons papular eruptions appear together with intensive itching and edema. It is a self-limiting ailment if no further infection is contracted. It is neither a communicable disease nor a fatal one, but the discomfort and the pathology of the infection contribute to its importance in the field of public health and veterinary medicine.

The disease is of geographical interest because it is a "Disease of the Place," as explained by Jarcho and Van Burkalow (1952). Normally the adults of the dermatitis-producing schistosomes are blood parasites of birds, or, in some instances, of mammals. The life cycle begins with the hatching of the eggs which are present in the droppings of infected animals. Suitable species of snails become infected upon contact with the miracidia hatched from the eggs, the snails serving as the intermediate host. After a proper incubation period in the snail, the infectious schistosome cercariae are issued into the surrounding water. Upon exposure to these cercariae, the bird or mammalian definitive hosts are infected and the parasites mature in the vascular system of the hosts. The life cycle is completed when the adult worms produce eggs. Humans contracting the cercarial dermatitis are only the result of accidental intrusion into the life cycle of these interesting parasites.

Therefore in any geographical area, the conditions responsible for the occurrence of cercarial dermatitis are: (1) the presence of birds infected with dermatitis-producing schistosomes, (2) the presence of the snail intermediate hosts with habits conducive to the acquisition of the disease, (3) ecological conditions favorable for the survival of both the hosts and the parasites, and (4) the presence of human beings engaged in an activity which exposes them to the infection. In many parts of the world these four basic factors are present, and, as a result, the distribution of cercarial dermatitis is worldwide (Cort, 1950; Kuntz, 1955).

In the Pacific area, the disease occurs at various locations on the west coast of the North American continent extending north to Alaska. It has been reported from Japan, the Federation of Malaya, Australia, and as far south as New Zealand. The Hawaiian Islands chain is not entirely free from the presence of this disease. The following report is an attempt to summarize from the literature[3] the important facts concerning the vectors and reservoir hosts of cercarial dermatitis and to emphasize the distribution and transmission of both fresh-water and marine schistosomes in various parts of the Pacific.

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[3] The survey of literature on cercarial dermatitis was concluded in July, 1957.

quiries on cercarial dermatitis: Dr. W. R. Giedt, chief of Division of Epidemiology and Laboratories, Department of Health, Washington; Richard F. Peters, chief of Bureau of Vector Control, and Benjamin Keh, assistant vector control specialist, Bureau of Vector Control, Department of Public Health, California; Ralph B. Williams, director of Division of Public Health Laboratories, Alaskan Department of Health; Curtiss M. Everets, Jr., director of Division of Sanitation and Engineering, Oregon State Board of Health; Dr. W. E. Martin, professor of zoology and chairman, Department of Biology, University of Southern California; and Dr. H. F. Hsu, research assistant professor, Department of Hygiene and Preventive Medicine, State University of Iowa. Special thanks are offered Dr. J. R. Audy, of the Institute for Medical Research, Kuala Lumpur, Federation of Malaya, for suggesting the preparation of this report and for the invitation to participate in this symposium, General Ecology of Man, Animal Reservoirs, and Vectors in Relation to Disease, at the Ninth Pacific Science Congress.

A. FRESH-WATER CERCARIAL DERMATITIS

1. *United States and Canadian Pacific Coasts*

The etiology of swimmer's itch was first elucidated by Cort in Michigan, U. S. A., in 1928. Since that time, it has been recognized that "nonhuman" species of schistosomes are responsible for many cases of swimmer's itch. In the United States and Canada, Jarcho and Van Burkalow (1952) indicated recently in their report that "the states most strongly affected are Michigan, Wisconsin, and Minnesota. A few clinical cases (cercariae not identified) have been reported from the neighboring states of Indiana, Illinois, Iowa, Nebraska, and North and South Dakota. In Canada the major areas are in Ontario, east of Georgian Bay; in Manitoba, in and near Lake Winnipeg; in Saskatchewan; and in Alberta." Other areas mentioned in the text include: British Columbia, Washington, Oregon, Quebec, New York, Maine, Nevada, Oklahoma, Alabama, Tennessee, and Florida. The disease is common in lakes and swamps along the flyways of migratory water fowls.

Recent data, together with previous reports relative to the following Pacific states, are given as follows:

a. *Washington*. Miller (1925, 1927) was first to describe the presence of schistosome cercariae in the northwestern United States. *Cercaria elvae* from *Lymnaea stagnalis* Linn. and *Cercaria tuckerensis* from *Planorbis* spp. were obtained from Trout Lake, San Juan Sound, Washington. Swimmer's itch in the Seattle area was shown by Hunter, Shillam, Trott, and Howell (1949) to be caused by two agents: (1) the cercariae of *Trichobilharzia ocellata* (La Valette) (=*C. elvae*) from *Stagnicola palustris nutaliana*, and (2) *Cercaria physellae* of *T. physellae* (Talbot) from *Physella propinqua* (Tryon). The former species of snail was found associated with reeds and other aquatic vegetation growing in the gravel and mud bottom of the lake. It was thought that the cercariae from infected snails were carried to shore by currents created by the wind blowing towards the beaches. A similar situation was known to have existed in Douglas Lake, Michigan (Cort, 1936).

The definitive hosts for these parasites in Washington have not been described, but, according to McMullen and Beaver (1945), *C. physellae*, whose definitive host was reported to be the blue-winged teal, *Querquedula discors* (Linn.), was also infective to pigeons, mallards, and canaries; however, a Caspian tern, chickens, domestic ducklings, and gulls could not be infected. The blue-winged teal is also the definitive host of *T. ocellata* (=*T. Kossarewi*), but McMullen and Beaver showed that canaries, domestic ducklings, mallards, and pigeons could be the experimental hosts. (Chicks and a tern were resistant.) Recently Farr and Blankemeyer (1956) described a new species of schistosome, *Trichobilharzia brantae*, whose definitive host was a Canada goose (*Branta canadensis* L.) from the Turnbull National Wildlife Refuge, Washington.

Macy (1952) demonstrated that *C. elvae* from *Lymnaea stagnalis* was the causative agent for dermatitis cases at Cascade Lake, Orcas Island. Macy (1955) further reported that dermatitis cases were found at a lake south of Tacoma, Washington, and from Lake Chelan and Goose Lake, Okanogan County, in eastern Washington. In spite of the fact that a large number of lakes in the state of Washington might have harbored schistosome-infected snails, Dr. W. R. Giedt, chief of Division of Epidemiology and Laboratories of the Department of Health, received only ten reports of cases in 1955 and ten during 1956 (personal communication, 1957). Since swimmer's itch is not a reportable disease, these statistics are probably incomplete. However, the preventive measures advocated by the health officers of Washington with regard to control and avoidance of the infection must have been successful, as is indicated by the low incidence of infections.

b. *Oregon*. Macy (1955) investigated schistosome dermatitis cases in the Blue Lake area near Troutdale, Oregon. The causative agent, *C. oregonensis* from *Physa ampularia*, was a schistosome species originally described by Macfarlane and Macy (1946). It is interesting to note that these infected snails occurred most abundantly on dead or decaying vegetation in lakes, ponds, and marshes. They laid their eggs in late summer and the next generation of snails matured enough to lay their eggs during the early spring. Snails infected during late summer could carry the schistosome through the winter; thus in the following spring and summer, infected snails of both the old and new generations might produce cercariae for a longer time and therefore possibly might cause a higher level of infection in humans.

c. *California*. Simmons, Martin, and Wagner (1951) reported that the first case of freshwater cercarial dermatitis in California was derived from an artificial lake at Alendra Park in Los Angeles County. Schistosome cercariae of three different types were obtained from *Physa osculans* (Haldeman) found in this lake. The taxonomic consideration of these cercariae is currently delayed because of the efficient control of the intermediate hosts by health officers (personal communication from Dr. W. E. Martin, 1957). Walker (1954) investigated a suspected outbreak of schistosome dermatitis at Twain Harte Lake, Tuolumne County, and found bifurcated cercariae coming from a mixture of *Physella* and *Lymnaea* snails. Taxonomic determinations of the schistosomes involved in these dermatitis cases are yet to be announced. Keh and Grodhaus (1957) reported swimmer's itch at a duck club in Yuba County. Cercariae of the "*ocellata*" group were obtained from *Physa* snails and later proven by experimental infection to belong to the *Trichobilharzia* group of bird schistosomes. As yet, there is no report of schistosome infected snails or cases of cercarial dermatitis in northern California.

d. *Canada*. As indicated previously, migratory water fowls such as the blue-winged teals are known to be the definitive hosts of dermatitis-producing schistosomes. Because of the many glaciated lakes and swamps which act as bird refuges, cercarial dermatitis in Canada is known to occur in many areas. Along the Pacific coast of Canada, only two species of dermatitis-producing schistosome cercariae have been reported. These cercariae were believed to be the agents responsible for a severe outbreak in 1950 at Cultus Lake, B. C. Edwards and Jansch (1955) described these two species as *Cercaria adamsi* and *C. columbiensis*, which have *Physa coniformis* Tryon as the intermediate host. The adult forms of *C. adamsi* are now designated as *Trichobilharzia adamsi* Edwards and Jansch, 1955. The definitive hosts for *C. adamsi* were believed to be wild water fowl because domestic ducks could be infected experimentally. The authors suggested that the close resemblance of *C. columbiensis* to the cercariae of *Gigantobilharzia huronensis* Najim would indicate that the pas-

seriform birds might be the natural definitive hosts for *C. columbiensis*. Goldfinches and cardinals are known to be the hosts for *G. huronensis*.

2. *Alaska*

Students interested in cercarial dermatitis have long suspected that the disease may be present in Alaska because this region is the home of many migratory water fowls (Gabrielson, 1952). Harkema (1953, 1954, 1955, 1957) reported in a series of papers that swimmer's itch occurred in the Fairbanks and Yukon Flat areas in the Alaskan interior. He made a study of the life history of one of the two schistosome cercariae obtained from infected snails in this area. A total of 80 bodies of water was investigated during the summers of 1952 and 1953, and 20 of these 80 harbored infected snails of the species *Lymnaea palustris*, *L. stagnalis*, and *L. auricularia*. The incidence of the infection in snails seemed to vary according to location and time of the year. Additional localities having infected snails were reported in 1957.

There were no extensive human infections derived from avian schistosomes in Alaska. Some cercarial dermatitis cases were observed in the Fairbanks area in the vicinity of Fort Yukon. Harkema (1957) used cercariae from the naturally infected *Lymnaea stagnalis* for experimental infections of ducklings, and the adult worm was identified as *Trichobilharzia alaskensis* Harkema, McKeever, and Becker. The other type of schistosome cercaria remains unidentified. However, two naturally infected baldpate ducks harbored adult schistosomes belonging to the genus *Trichobilharzia*. So far there has not been an extensive study of the bird definitive hosts for schistosomes in Alaska.

Gabrielson (1952), in discussing Alaskan waterfowls and their management, stated that "Alaska has breeding populations of the Whistling Swan, Emperor Goose, White-cheeked Goose, Lesser Canada Goose, Cackling Goose, White-fronted Goose, and Black Brant. It also provides suitable nesting habitats for impressive numbers of Pintails, Mallards, Green-winged Teals, Baldpates, and Shovelers, among shallow water ducks." He thinks that the Yukon delta is not only the most important breeding area in Alaska, but one of the great waterfowl production units of the continent. The green-winged teals, pintails, and baldpates, which have an extensive continental breeding range, are less affected by man-made changes in Alaskan topography than are the other species of waterfowls. Therefore in the future, the baldpates, green-winged teals, and pintails are more likely to be implicated as reservoir hosts for the distribution of avian schistosomes.

3. *Mexico and El Salvador*

Possible fresh-water cercarial dermatitis from Lake Texcoco near Mexico City was reported by Cort (1950), but so far is unconfirmed. As far as this reviewer is aware, the only cases of swimmer's itch south of Mexico along the Pacific were those contracted from Lake Coatepeque, El Salvador, and reported by Reyes in 1944. According to Cort's account of the investigation by Reyes, the exact etiological agent was not identified. Jarcho and Van Burkalow (1952) reported that a blue-winged teal captured in this area had migrated from Douglas Lake, Michigan—the area where Professor W. W. Cort first found the cause of swimmer's itch. These blue-winged teals, according to these authors, migrate along the Mississippi River and the Atlantic coast flyways. The wintering grounds involved a large geographical area ranging from southern Florida, Texas, California, through Central America to parts of the northern South American continent, while the breeding grounds extend from the Great Lakes region westward to the Rockies and as far north as northwestern Canada.

Gabrielson (1952) showed that certain species of migratory fowls did migrate as far south as Mexico and that they could be recovered in British Columbia and the states of

Washington, Oregon, and California. Thus it is no surprise that swimmer's itch along the Pacific coast has been reported from Alaska to El Salvador.

4. *Japan*

On the western side of the Pacific Ocean, the Japanese have recognised for over 50 years the existence of a dermatitis of unknown etiology which occurred among farmers inhabiting land near Lake Shinji, Shimane Prefecture. The dermatitis is called "koganbyo" or "lakeside disease" (Hunter, Ritchie, and Tanabe, 1951).

These authors reported that Tanabe in 1947 was able to produce experimental dermatitis using the furcocercous cercariae from a species of snail now known as *Polypylis hemisphaerula* Benson. This observation was confirmed and subsequently the definitive hosts were determined. They are the large starling (*Spodiopsar cineraceus* Tamminck), the sparrow (*Passer montanus saturatus* Stejnerger), and the wagtail (*Motacilla* (*Motacilla*) *grandis* Sharpe). The adult of this species of schistosome is designated as *Gigantobilharzia sturniae* Tanabe, 1948.

The snail intermediate hosts (with 3–10 per cent infections) were commonly found on submerged vegetation or mud of rice fields and irrigation canals. Dermatitis began in mid-May and reached a peak about mid-July to mid-August, and the degree and intensity of the infection appeared to be correlated with the aquatic activity of the rice farmers. Surveys made of other areas in Japan indicated that both the bird definitive hosts and the snail intermediate hosts were widespread. Aside from Lake Shinji, the cases of dermatitis in the farmers of Mie Prefecture as well as those of Aichi Prefecture were believed to be caused by the cercariae of *G. sturniae* (Oda, 1956*a*, 1956*b*). It is possible that there are other areas in Japan where koganbyo exists.

Yamaguti (1941) reported the presence of avian schistosomes in Japanese birds and described two new species: *Trichobilharzia corvi* (Yamaguti, 1941) McMullen and Beaver, 1945, from *Corvus corone corone* Linn., and *Ornithobilharzia emberizae* Yamaguti, 1941, from *Emberiza sulphurata* Temm. et Schleg. However, it is not known if these parasites are capable of producing cercarial dermatitis.

An interesting report was made by Ito (1956) on a brackish water furcocercous cercaria from a snail intermediate host, *Tympanotonus microptera* (Kiener) found in Chiba Prefecture (Tokyo Bay). The author named it *Cercaria tympanotoni* and suggested that on morphological grounds this cercaria might be dermatitis producing. Because of an insufficiency of snails infected with this parasite, no experimental data were obtained. However, this record indicates, for the first time in Japan, that perhaps in a brackish water environment there is a possibility of humans contracting cercarial dermatitis.

5. *China and Formosa*

There are no reported cases in man of avian cercarial dermatitis in China or Formosa. However, two species of bird schistosomes have been found in China; *Ornithobilharzia odhneri* Faust, 1924, from the Asiatic curlew, and *Ornithobilharzia hoepplii* Tang, 1951, from Swinhoe's snipe.

In Formosa, one species of avian schistosome, *Trichobilharzia yokogawai* (Oiso, 1927) McMullen and Beaver, 1945, has been isolated from the duck. The "nonhuman zoophilic" strain of *Schistosoma japonicum* in Formosa has been regarded as a dog strain, because there is a high incidence (62 per cent) of natural infections in dogs but no known occurrence in humans (Hsu *et al.*, 1954). In order to determine the effectiveness of this strain in causing cercarial dermatitis and other symptoms in man, Hsu and Hsu (1956) tested the infectivity of this strain of *S. japonicum* on five human volunteers. The cercariae of *S. japonicum* did cause the sensation of itching and the development of a rash or papule. There were systemic clinical signs, such as abdominal pain, headache, nausea,

anorexia, chest pain, and general malaise, which are normally absent from avian schistosome cercarial infection. Nevertheless, the Formosan strain of *S. japonicum* did not develop to maturity in the volunteers and consequently did not result in a typical schistosomiasis. There were no experiments on repeated infections in these volunteers to determine hypersensitivity reactions.

Japanese investigators had long suspected that *S. japonicum* ("human strain") might cause cercarial dermatitis. In 1909, Kobayashi studied the relations between "kabure," endemic dermatitis, and schistosomiasis japonica (Faust, 1924*b*). Recently Ishii and Ogawa (1952) reported that it was possible to produce dermatitis in rats with the cercariae of *S. japonicum* ("human strain") by repeated exposure to the cercariae. Characteristic lesions included macules, papules, erythema, edema, and vesicles. However, Hunter and his associates (1956), using mice, hamsters, and rabbits as experimental animals, were unable to repeat these results or similar findings reported by other workers and so concluded that the selection of the proper experimental hosts for studies on immunologic responses to schistosome infection is of critical importance.

6. *Malaya and the Philippines*

Buckley (1928) demonstrated that the dermatitis known as "sawah itch" in Malaya was caused by the cercariae of *Schistosoma spindale* Montgomery, 1906, obtained from *Planorbis pfeifferi*. Normally *S. spindale* causes serious schistosomiasis in mammals such as cattle, sheep, goats, horses, antelopes, and water buffaloes in India, South Africa, and Sumatra.

Another example of a mammal-infecting schistosome which causes dermatitis in man is *Schistosomatium douthitti* (Cort, 1914). This organism is found in the United States as a parasite in deer, mice, and muskrats.

A fruitful field for research would be a comparative study to determine the differences between the mammalian schistosomes, which can cause dermatitis in man (like *S. spindale*, *S. douthitti*), and the dog-infecting strain of schistosome (*S. japonicum*), which apparently does not cause natural cercarial dermatitis (koganbyo) among Formosan farmers.

Recently a bird schistosome belonging to the "*elvae*" group of cercariae was described by Sandosham (1953) as the cause of sawah itch in the Ayer-Lunging district, Negri Semilan, and the intermediate host was identified as *Lymnaea rosseana* Mabille. This dermatitis-producing cercaria was named *Cercaria Malaya I*, and an unsuccessful attempt was made to infect chickens with it.

Cercarial dermatitis in man in the Philippines has not been studied to any extent, probably because of the intense efforts on the part of the medical scientists to control the spread of schistosomiasis japonica. Tubangui (1947) listed *Austrobilharzia bayensis* Tubangui, 1933, as a schistosome from the mesenteric vein of a snipe, *Capella gallinago gallinago*. This finding is encouraging for those who are interested in further host examinations for the presence of avian schistosomiasis, especially in the sea and shore birds of the Philippines.

7. *New Zealand*

According to Macfarlane (1944, 1949*a*, 1949*b*) the reports of swimmer's itch derived from lakes Hawea, Te Anau, Alexandrina, and Rotoiti, and from several lakes in the Rotorua district, have not been confirmed. However, Macfarlane recovered schistosome cercariae from snails in lakes Wanaka, Hayes, and Wakatipu in the South Island. The etiological agent causing experimental dermatitis has been described and named *Cercaria longicauda* Macfarlane, 1944. The snail intermediate hosts are: *Myxas ampulla*, *M. arguta*, and *Limnaea alfredi*. The molluscan hosts in Lake Wanaka lived in beds of the pond weed *Myriophyllum robustum* and also on *Isoetes* spp., *Juncus* spp., and *Potamogeton polygonifolium*. In lakes Wakatipu and Hayes there were

fewer snails, associated with the weed *Ranunculus fluitans*. The definitive host, the teal *Fuligula novae zealandii*, rested on the weed beds through the summer and fed on the lake floor. The adult stage of *C. longicauda* has not been studied.

8. *Australia*

Fresh-water cercarial dermatitis has been known in Australia since Johnston (1941) first reported its presence in the Murray Valley swamps. He surmised that *Cercaria parocellata* Johnston and Simpson, 1939, from *Limnaea lessoni*, on morphological grounds, might be the parasite involved. Macfarlane (1952) made a further investigation and obtained reports of dermatitis at Albury, Birri, Loxton, Mannum, Murray Bridge, and Tailem Bend near Adelaide. According to this author, at all of these places the river spread into areas of shallow water, which allowed the molluscan hosts to build up a population in close association with water birds. He believed that most of the infection of humans occurred during periods of shallow water.

Swimmer's itch was also known to be present in Western Australia. Lake Bumbleyung in the Wagin region appeared to be the important area although other smaller lakes had also been the source of the infection. The third area in Australia suspected to have cercarial dermatitis was Narrabeen Lake, a small shallow coastal lake near Sidney. It has been reported since that these cases were caused by marine species of bird schistosomes, because this lake has an outlet to the Pacific Ocean and the water may not be fresh.

Macfarlane reported that the snail intermediate host *L. lessoni* occurred in both the Murray Swamp area and the Wagin region. Whether or not *Cercaria paracellata* was involved in both areas is not known. In the Murray River, *L. lessoni* lived in the often stagnant backwaters.

Johnston (1941) suspected that the black swan was the definitive host for *C. parocellata*, but confirmation has not been made. *Cercaria jaenschi* was reported by Macfarlane (1952) not to penetrate the skin of man even though it was a schistosome cercaria. Furthermore, the antigen prepared from these cercariae produced smaller and less persistent skin reactions than did the *C. parocellata* antigen.

B. MARINE CERCARIAL DERMATITIS

1. *California and Mexico*

Extended studies of marine snails as possible agents in the transmission of cercarial dermatitis were not made until several years after World War II. However, as early as 1942, Penner mentioned that the marine gastropods might be infected with dermatitis-producing schistosome cercariae. In 1950, he described as a new species *Cercaria littorinalinae* from the marine snail *Littorina planaxis* Philippi, which were collected along the rocky shores of the Coronado Islands in Mexico and from Bird Rock near La Jolla, California. The natural definitive host was the Wyman western gull (*Larus occidentalis wymani* Dicky and van Rossem). Penner (1953*c*) was able to infect experimentally with this species of cercaria the following birds: brant, cormorant, black-crowned night heron, western gull, budgerigar parakeet, Australian zebra finch, domestic pigeon, red jungle fowl, and linnet. The domestic duck did not appear to be susceptible to the infection. The adults of *C. littorinalinae* belong to the genus *Austrobilharzia* (Johnston, 1917) and have been given the name *Austrobilharzia littorinalinae* by Penner although the taxonomic description is still unavailable. This species of schistosome is therefore distinct from *A. variglandis* (Miller and Northup, 1926) Penner, 1953 (= *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951).

In the San Diego area, this marine species of bird schistosome has not been officially incriminated in cases of swimmer's itch although Penner (1950) did mention that he experienced the development of typical lesions after wading in sea water in this area

while collecting the snail intermediate hosts. Cercarial dermatitis can be produced experimentally with *C. littorinalinae*.

Recently Gradhaus and Keh (unpublished data, 1957) reported cases of swimmer's itch occurring on the beaches of the city of Alameda, bordering San Francisco Bay. *Nassarius obsoletus*, a marine snail intermediate host for *A. variglandis* on the Atlantic coast, was also the intermediate host in California. It was believed that the snails were introduced into this area together with the oysters that had been planted in the bay. The schistosome cercariae from these snails were identified as those of *A. variglandis*.

2. *Hawaiian Islands*

Chu (1952) first reported the presence of a dermatitis-producing schistosome cercaria from the marine snail *Littorina pintado* Wood in two bird refuges on small offshore islands near Oahu, known as Bird Island (Moku Manu) and Rabbit Island (Manana). The snails infected with schistosomes were found only in the sea benches where there were many small tidal pools constantly receiving a sea spray. Collectors of the snails from the sea benches of Rabbit Island experienced itching and, in some cases, developed typical schistosome papule lesions on their feet. It may be added that the distribution of infectious cercariae seems to be limited to the bird refuges, because Chu and Cutress (unpublished data, 1954) were unable to find schistosome-infected snails inhabiting any of the swimming beaches on the major islands of Oahu, Maui, Kauai, and Hawaii. The life history of this parasite has been reported by Chu and Cutress (1954). The natural definitive host for the adult stage was the ruddy turnstone (*Arenaria interpres interpres*), a shore bird which winters in the Hawaiian Islands. Chicks, ducks, and the sooty and noddy terns could be infected experimentally.

After a study of the morphological characters of the adult stage of the schistosome in the naturally infected turnstones as well as in the experimentally infected animals, the authors concluded that this species was the same as the species of schistosome reported by Stunkard and Hinchliffe (1951, 1952) from the eastern United States along the Atlantic Ocean, i. e., *Austrobilharzia variglandis*.

Penner (1953*b*) reported that the red-breasted merganser (*Mergus serrator* L.) was the natural definitive host for *A. variglandis* in the Atlantic coast. Munro (1944) states that this species of bird had been reported only as an occasional visitor to the Hawaiian Islands. However, Richardson and Fisher (1950) do not list the red-breasted merganser as a species found in the Hawaiian bird refuges. It can also be added that the western gull, a natural definitive host for *A. littorinalinae* in California, is not a species generally seen on Oahu.

The migration routes for shore birds in the Pacific Ocean have been studied by Baker (1951). On the basis of sight records, specimen collection, known statistics of breeding and wintering, and from a study of maps of the region, he established the following three flyways: (1) the Asiatic-Palauan, (2) the Japanese-Marianan, and (3) the Nearctic-Hawaiian. For each of the flyways the author listed the names of the regularly visiting and the uncommonly visiting shore birds. Of the 35 species mentioned, only the ruddy turnstone and the golden plover (*Pluvialis dominica fulva*) were the ones common to all of the three flyways. In the experience of the Hawaiian investigators, the golden plover was never found to be infected with the schistosome, in contrast to the ruddy turnstone.

King (1955) listed six major groups of sea birds found in the central Pacific Ocean. These are: (1) the albatrosses, (2) the shearwaters and petrels, (3) the terns, (4) the frigate birds, (5) the boobies, and (6) the tropic birds. Gulls and jaegers were listed as being occasionally seen. Chu and Cutress (unpublished data, 1954) did not encounter a single case of natural infection with schistosomes in limited examinations of sea bird pop-

ulations. The exact relation of the sea birds to the problem of transmission and reservoir hosts in Hawaii has yet to be determined.

The snail intermediate host, *L. pintado*, uncommon in Micronesia and apparently limited in its range to the northern Pacific islands, has been reported by Demond (1957) to exist in southern Japan, the Ryukyus, Bonins, northern Philippines, Marianas, Marshalls[4], Johnston Island, and Formosa. The experimental snail intermediate host used by Chu and Cutress, *Littorina scabra* L., has a much wider geographical range: from east Africa through the Indian and Pacific oceans to Hawaii and Tahiti, and from the Ryukyus, the Philippines, and Marianas, south to northern Australia. Since both the definitive and intermediate hosts for *A. variglandis* are widely spread in the Pacific area, one may surmise that marine schistosome infections of birds and snails may not be limited to the Hawaiian Islands and California and that with further investigation other areas might prove to be infected.

In determining the casual agent of swimmer's itch, or cercarial dermatitis, it is not sufficient to find schistosome-infected snails in the area because of the possible presence of other etiological agents which cause similar skin lesions. "Swimmers' Itch" (or "Pearl Harbor Itch") cases reported by Arnold and Bonnet (1950) are now suspected to have been cases of marine hydroid dermatitis, because the discovery of a medusa (*Sarsia* sp.) was made in the same location which Arnold and Bonnet reported to be a source of the dermatitis. This finding, together with the absence of schistosome-infected snails in the areas under study, suggests that these "Swimmers' Itch" cases of Arnold and Bonnet were not of cercarial origin (Chu and Cutress, unpublished data).

It is necessary, however, to point out that in the Atlantic coast, (such as Mill cove in Maine, Narragansett Bay in Rhode Island, and Long Island Sound, N. Y.) cercarial dermatitis was reported to be associated with clam digging (Orris and Combs, 1950; Sindermann and Gibbs, 1953). The intermediate host is a mud snail, *Nassarius obsoletus* (*Nassa obsoleta*), which is also an intermediate host for the marine schistosome in California. This species is not found in Hawaii as Edmondson (1946) listed *Nassa sertum* Bruguiere as the only local species. In several surveys made during 1951–54 by the Hawaiian investigators, the few bottom-dwelling gastropods from West Loch, Pearl Harbor, were never found to be infected with schistosome parasites.

Demond (1957) listed *Nassarius papillosus* (Linn.) as occurring in Hawaii. As yet, there is no report indicating that this or any other species of *Nassarius* is an intermediate host for schistosome in the central and south Pacific.

An example of the difficulty of determining the etiological agent for dermatitis caused by marine microorganisms is that reported by Strauss (1956) for the "Seabather's Eruption" in Guantanamo Bay, Cuba. This investigator was unable to find any snails infected with schistosomes. Serological tests of the patients showed negative evidence for the presence of cercarial dermatitis. A more fortunate experience than that reported by Strauss was given by Chu and Cutress (1955). Many construction workers contracted dermatitis when in contact with sea water in Hilo Bay, Hawaii. Although in this area, *Littorina pintado* and the ruddy turnstone were seen, no schistosome-infected snails were located. Instead, a marine hydroid, *Syncoryne mirabilis* (Agassiz, 1852), was found growing profusely on the surface of the submerged rocks near the site of construction. By chemical treatment, the hydroids were destroyed and the dermatitis was controlled.

Most of the conclusions incriminating marine schistosome cercariae in cases of derma-

[4] In 1955, at Eniwetok, C. E. Cutress found *L. pintado* and ruddy turnstones in the area, but among the specimens examined by him no schistosomes were found (personal communication, 1957).

titis have been based upon circumstantial evidence. The urgent need in diagnosing the cause of naturally occurring dermatitis is a technic (e. g., biopsy tests) which would yield direct evidence about the infective agent, so that proper control methods could be formulated.

3. *Australia*

Bearup (1955, 1956) showed that schistosome cercariae occurred in a marine snail, *Pyrazus australis* Quoy and Gaimard, in Narrabeen Lake, near Sydney, N. S. W. The cercaria was first identified as *Cercaria variglandis* later Miller and Northup, subspecies *pyrazi*, but was determined to be the cercaria of *Austrobilharzia terrigalensis* Johnston, 1917. The seagulls (*Larus novae-hollandiae*) from the Sydney district were heavily infected.

The incidence of schistosome infection in *P. australis* from Narrabeen Lagoon was 4–6 per cent, the higher level occurring during the hot months at the end of the year. Cercariae from these infected snails would infect young seagulls, budgerigars (*Melopsitacus undulatus*), and pigeons (*Columba livia*), but not domestic ducks or mice.

From Florida, U. S. A., several marine schistosome cercariae have been described (Hutton, 1952; Leigh, 1955; Penner, 1953*a*). These species are distinct from one another and different from *A. variglandis*. However, the extreme similarity in morphology of the three species, namely, (1) *A. variglandis* from the Atlantic, Pacific, and Hawaiian areas, (2) *A. littorinalinae* from southern California and Mexico, and (3) *A. terrigalensis* from Australia, raises the interesting question of whether or not they are actually one species with minor variations because of adaptation to the intermediate and definitive hosts in widely separated geographical areas.

DISCUSSION

Cercarial dermatitis is of worldwide distribution, and probably there are other areas where suspected cases of swimmer's itch occur but where no investigations have been made. In the Pacific Ocean area, many islands in the Polynesian, Micronesian, and Melanesian groups are worthy of exploratory studies for the presence of bird schistosomiasis. Likewise, other geographical areas such as China, Formosa, Indonesia, Viet Nam, the Philippines, and Thailand have yet to be investigated fully.

In the North American continent, swimmer's itch has been widely studied, yet data on schistosome infections in migratory birds are still limited. Most of the information on definitive hosts has been obtained through experimental infection. As a result, the question is frequently asked as to the degree of natural infection with schistosomes in other types of birds. In this connection, the problem of host susceptibility has been studied by many investigators. In our own experience, the marine schistosome adults found in the ruddy turnstone developed well in the sooty and noddy terns, but in chicks and ducks the adults did not remain viable for an extended period and the pathology was not entirely comparable to that in the natural host. This differential susceptibility between species and subspecies (or strains) of the birds is a challenging problem to workers who are interested in immunology. Such a study is important in relationship to speciation and distribution of both the fresh-water and marine schistosomes in birds.

Specificity of a trematode parasite for a host may be directly correlated with the host diet. An example of this correlation was reported by Oguri and Chu (1955) for the infection of domestic ducks by the cloacal trematode *Parochis acanthus* (Nicoll, 1906) Nicoll, 1907. The natural definitive hosts for this parasite are sea birds. When the infectious cysts of *P. acanthus* were given orally to mash-fed ducks, there were no infections in these experimental hosts. However, if the ducks were fed with squids from the sea, instead of the mash diet, it was possible to establish the

infection in them. This variation in host susceptibility caused by a change in diet suggests that seemingly contradictory results from host experimental infections for bird schistosomes may be due to differences in the diets fed the experimental hosts by the various investigators.

Substantial data, on the other hand, have accumulated during the past several decades regarding the bionomics of the intermediate hosts of the dermatitis-producing schistosomes. These data are chiefly concerned with (1) the habits of the snails, such as their food requirements, reproductive rates, adaptation to surroundings, and association with other plant and animal life, (2) the environmental conditions such as changes in pH and salinity in water, variation in temperature and amount of sunlight, and (3) susceptibility of the other species of snails in the locality to infection by the schistosome miracidia. This information is essential for understanding the reasons why the disease exists in specific localities. Many reports also indicate that an intimate association between the infected birds and the snail intermediate hosts is a necessity before cercarial dermatitis can be established as a "Disease of the Place."

For fresh-water snails, cultivation in the laboratory has not been a problem, but for marine snails, technics for their cultivation are yet to be developed. The bionomics of marine snails is a fascinating subject. Edmondson (1946) stated that: "A specimen of *Littorina pintado* remained attached to the plastered wall of the Marine Biological Laboratory, Honolulu, for nearly a year. When it was returned to sea water it regained its activity in a few minutes." Attempts are now being made to breed *Littorina* snails in our laboratory for use in experimental work on schistosomes.

The investigation of all phases concerned with marine cercarial dermatitis is indeed a green pasture for those who are interested in diseases associated with a marine environment. We encourage investigators in the Pacific area to participate in this field of study.

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